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TOXINS AND TISSUE RESPIRATION [1]

Paul J. Allen

It is now generally recognized that the symptoms of a parasitic disease arise largely from an interchange of chemical substances between the invading parasite and its host. This concept was a logical development after the firm establishment of microorganisms as the causative agents of many diseases, and affords an experimentally useful approach to the study of parasitism. In animal pathology, it has led to remarkable advances in the understanding and control of disease. The failure, until recently, to apply this concept as extensively in plant pathology may perhaps be ascribed to the focus of attention in the case of plants upon populations rather than individuals. In the plant as well as the animal, however, it is the individual which suffers disease, and to understand the cause and course of a disease attention must be given to the response of the individual plant to a foreign organism.

The concept of chemical substances as causative agents of disease implies the production by the parasite of diffusible metabolites having injurious effects upon the host. Whether these effects appear as morphological or as physiological changes, they must ultimately be traced to changes in metabolism. This paper will discuss some of the changes in respiration which may reasonably or definitely be attributed to the action of diffusible metabolites. Since respiration is the process which provides the main source of available energy and of raw materials for synthesis and maintenance, it is of basic importance to the health of the individual. It must, however, be recognized that profound changes in the utilization of energy may occur without corresponding changes in the overall process of respiration; and conversely, that major changes in respiration do not necessarily reflect equally important changes in the utilization of energy. It is therefore certain than an eventual understanding of the role of metabolic changes in the development of disease will involve a study of isolated links in the chain of energy conversions as well as a study of the overall process.

Respiratory changes in diseased tissues have been reported by many investigators. Furthermore, the changes observed are almost always increases. The main part of this paper will be devoted to evidence for the occurrence of augmented respiration as a response to toxic action, and to an analysis of the causes underlying its development. The inhibitory action of some higher plant and microbial toxins on the respiration of microorganisms will then be considered.

THE OCCURRENCE OF AUGMENTED RESPIRATION.—In storage organs infected with fungi and bacteria increased O_2 uptake, CO_2 production, and tissue temperature have all been reported. Representative data for several tissues of this type are summarized in Table 1. All the figures in this table are based upon a comparison with healthy tissues treated identically except for the exposure to a parasite or its metabolic products. In Weimer and Harter's experiments (51), the rate of CO_2 production by half roots of sweet potatoes infected with *Rhizopus tritici* rose above the controls during the first day and was as much as 9 times that of the noninfected half during the second day. Equally large increases in the infected tissues of Irish potatoes after inoculation with *Bacillus phytophthorus* were reported by Eglits (13). Marked rises in temperature (up to 0.136°C as compared with a normal of 0.005°C) were reported by Eglits for this same disease, not only in the infected zone of the tuber, but also at points several centimeters away. Hyperthermy has also been found by Fischer and Gaumann (14) in tubers infected with *Phytophthora infestans* and *Fusarium solani*. Hellinga (22, 23) carried out extensive studies in an attempt to identify an active principle responsible for increased respiration. Using thin slices of tubers, he found that O_2 uptake and CO_2 production were increased by extracts of the mycelium of *Gibberella Saubinetii*, which causes a soft rot of the tubers, and that smaller increases were produced by culture filtrates and by extracts of infected tubers. The fungus extracts were still active when diluted 10^5 times. The active substance was water soluble, nonvolatile, and heat stable and was found in extracts of each of several different fungi which he tested. His conclusion that it might be pantothenic acid, though plausible, has not been confirmed.

In 1941, Biale and Shepherd (8) reported on the augmented respiration of citrus fruits elicited by other fruits infected with *Penicillium digitatum*, and showed that it was caused by a volatile substance from the rotting fruit. Respiratory increases had been found earlier by Denny (12) to result from exposure of lemons to very small concentrations of ethylene, a compound which was later identified as a volatile product of the rot fungus *Penicillium digitatum* by Young, Pratt, and Biale (56). Their work represents one of the clearest demonstrations of the chemical identity of a fungus toxin eliciting the symptoms of metabolic derangement in diseased tissues, and will be discussed further in a later section. It is noteworthy that the respiration-stimulating substance of *Penicillium digitatum* is also a normal metabolic product of many fruits and plays an important part in their ripening. At the time of ripening, there is a rise in respiration associated with an autogenous rise in ethylene production, after which further addition of ethylene no longer stimulates respiration.

The preceding examples indicate the effects of facultative parasites and their products on the respiration

[1] An invitational paper presented at the Symposium on Physiology of Parasitism at the 44th Annual Meeting of The American Phytopathological Society in Ithaca, N. Y., September 9, 1952.

TABLE 1.—*Enhanced respiration of infected storage tissues and tissues affected by metabolic products of parasitic fungi and bacteria. Figures in % of control, except temperature, which is °C. above control*

Plant and Organ	Parasite or Agent	Hrs. after infection or exposure to agent	O_2 Uptake (or Temperature)	CO_2 Production	Reference
Sweet potato—half root	*Rhizopus tritici*	0–24	....	121	Weimer & Harter (51)
Sweet potato—half root	*Rhizopus tritici*	24–48	....	944	"
Irish potato—infected zone	*Bacillus phytophthorus*	130	....	904	Eglits (13)
Irish potato—infected zone	*Bacillus phytophthorus*	130	0.136°C	....	Eglits (13)
Irish potato—2.0 cm from infected zone	*Bacillus phytophthorus*	130	0.072°C	....	Eglits (13)
Irish potato—4.0 cm from infected zone	*Bacillus phytophthorus*	130	0.032°C	....	Eglits (13)
Irish potato—whole tuber, infected zone	*Phytophtora infestans*	?	0.04°C	....	Fischer & Gaumann (14)
Irish potato—whole tuber, infected zone	*Fusarium solani*	?	0.02°C	....	Fischer & Gaumann (14)
Irish potato—0.5 mm. slices	*Gibberella Saubinetii*-extract	0–1	208	....	Hellinga (23)
Irish potato—0.5 mm. slices	*Gibberella Saubinetii*-filtrate	0–1	134	....	Hellinga (23)
Irish potato—0.5 mm. slices	*Gibberella Saubinetii*-extract of infected tuber	0–1	178	....	Hellinga (23)
Lemon—whole fruit	*Penicillium digitatum*-emanation	144	....	190	Biale & Shepherd (8)
Lemon—whole fruit	Ethylene $1:10^4$	48	....	290	Denny (12)
Lemon—whole fruit	Ethylene $1:10^5$	48	....	147	Denny (12)
Pear (Anjou)—preclimacteric fruit	Ethylene	144	....	200	Hansen & Hartman (20)
Pear (Anjou)—postclimacteric fruit	Ethylene	144	....	104	Hansen & Hartman (20)

of storage organs. Equally marked rises in O_2 uptake and CO_2 production have been found in stems and leaves infected with strict parasites. Some representative data for plants infected with rusts, smuts, and powdery mildews are summarized in Table 2. Diseases caused by the ectoparasitic powdery mildews have provided the most suitable material for distinguishing between the respiration of the host and the parasite. Dusting with sulfur, which kills the mildew, does not appreciably alter the respiration of mildewed leaves (55, 43). Allen and Goddard (3), working with wheat leaves infected with powdery mildew, found by mechanical removal of the mildew mycelium, by differential poisoning of the mildew respiration with sodium azide, and by measurement of the respiration of the intact mildew isolated on epidermal strips, that most of the increased respiration was occurring in the non-invaded tissues of the leaf (Table 2). Smaller increases in anaerobic CO_2 production reported by Allen and Goddard (3) and by Sempio (46) can be attributed entirely to the host tissues, since the mildew itself produces no CO_2 anaerobically.

It is evident from these data (a) than an increase in respiration is a widespread property of diseased tissues; (b) that most of the increase can be attributed to the host cells; and (c) that the increase is elicited by diffusible substances originating from the parasite. What is the mechanism whereby these increases in respiration are brought about? What happens is that mature cells respiring at a relatively slow rate are affected by substances which make them respire more rapidly. In other words, the rate of respiration in healthy tissue is held in check, and the restraining factors which keep it down are partly or wholly abolished by the toxins of a parasite. In order to gain some insight into the mode of action of these substances, we must, therefore, consider the factors which may determine the normal regulation of respiratory rate. Briefly, these include a) access of O_2; b) the quantity of each enzyme participating in the process; c) substrate concentration; d) freedom of the enzymes involved to combine with their substrates and with any necessary co-factors; and e) the availability of co-factors and acceptors.

CAUSES OF INCREASED RESPIRATION.—*Access of O_2.*—It is unlikely that improved access of O_2 can account for much if any of the augmented respiration described above, since increasing the external O_2 tension usually has very little stimulating effect upon respiration. In Hellinga's experiments with thin slices of tuber and in measurements with leaves, the possibility of limiting O_2 diffusion is particularly slight.

Enzyme concentration.—Little information is available concerning the enzymes of diseased plant tissues as compared to healthy tissues of the same kind. Assays for enzyme activity give only a suggestion of the quantity of enzyme unless careful precaution is exercised to avoid confusing differences in activity with

TABLE 2.—*Enhanced respiration of leaf and stem tissues infected with strict parasites. Figures as percentage of control*

Plant and Organ	Parasite and Treatment	Days after Infection	O_2 Uptake	CO_2 Production	References
Euphorbia Peplus–leaves	*Melampsora helioscopiae*	?	273	217	Maresquelle (36)
Althea rosea–leaves	*Puccinia Malvacearum*	?	149	145	Maresquelle (36)
Clover–leaves	*Uromyces fallens* on 10% sucrose	7–8	—	285	Yarwood (55)
Wheat–leaves	*Ustilago tritici*	?	—	121	Kourssanov (31)
Wheat–stem	*Ustilago tritici*	?	—	140	Kourssanov (31)
Clover–leaf	*Erysiphe polygoni* on 10% sucrose	7–8	—	190	Yarwood (55) Pratt (43)
Wheat–1st leaf	*Erysiphe graminis*	9	280	—	
Wheat–1st leaf	*Erysiphe graminis*	11	434	—	Sempio (46)
Wheat–1st leaf	*Erysiphe graminis*	7	466	—	Allen & Goddard (3)
Wheat–1st leaf	*Erysiphe graminis*–parasite removed	7	377	—	Allen & Goddard (3)
Wheat–1st leaf	*Erysiphe graminis*–parasite respiration inhibited with azide	7	400	—	Allen & Goddard (3)

differences in quantity. Reports of increased catalase in rusted wheat leaves (9) are subject not only to this uncertainty, but also to an uncertainty as to whether the extra activity was in the host or the parasite. Increases in catalase (38, 30) and peroxidase (38) not attributable to the inciting parasite, have been reported in gall tissues, and tyrosinase activity is also frequently high in gall tissues. We have found ascorbic acid oxidase activity of young corn smut galls to be 2–3 times as high as that of comparable healthy kernels. This is of particular interest in view of the known production of indoleacetic acid by the corn smut fungus (53), the higher auxin content of smut galls (37), and Newcomb's finding (40) that auxin causes an increase in ascorbic acid oxidase in tobacco pith tissue. None of these enzymes, however, has a well-established role in respiration, and though changes may exist in diseased tissues, they may have little significance in explaining an increased respiration.

Greater amylase activity has been reported for wheat leaves infected with rust (9) and with powdery mildew (45), and Sempio (45) has found increases also in lipase activity. In all of these reports the increases were said to occur in the earlier stages of the disease and were later followed by marked decreases.

These limited reports provide very little basis for evaluating the possible inportance of enzyme formation as a cause of higher respiration. Such investigations should center particularly on enzymes known to be involved in respiration, such as cytochrome oxidase, dehydrogenases and transphosphorylases, and should be correlated with the actual respiratory changes in a given tissue.

Substrate concentration.—Considerable information is available on the changes in substrate concentration and their possible relation to increased respiration. Weimer and Harter (51) found that sucrose decreased and reducing sugars sometimes increased a little, sometimes decreased in sweet potatoes infected with *Rhizopus tritici*. The concentration of these sugars could not, therefore, provide an adequate explanation for the large increases in respiration observed. A substrate effect was definitely eliminated as a possible source of the respiratory stimulation observed by Hellinga in potato tuber slices. Much smaller effects were obtained from sucrose, and the total carbon content of diluted extracts was far below that of the extra CO_2 evolved. The action of ethylene on fruit respiration is likewise difficult to relate to an increase in soluble substrates. Neither the ethylene-induced rise (19) nor the analogous climacteric rise in respiration is paralleled by sucrose or reducing sugar concentration (6).

Allen (2) investigated the relation between changes in respiration and carbohydrate in wheat leaves infected with powdery mildew, and found in this case that major increases in starch, sucrose, and reducing sugars accompanied the rise in respiration. The closest correlation was found between reducing sugar concentration and rate of O_2 uptake, with an R.Q. of 1.0 indicating that carbohydrate was being oxidized. The reducing sugar is probably free and not phosphorylated; Miss Weisel, working in our laboratory, has found no increase in hydrolyzable organic phosphates. Glucose supplied to healthy wheat leaves, however, causes much smaller increases in respiration than those elicited by mildew infection, suggesting that other changes besides the substrate level are instrumental in bringing about very high respiratory rates. A similar conclusion is evident from Yarwood's experiments (55) with mildew and rust infected clover leaves. Working with isolated leaflets floated on 10 per cent sucrose solutions, he found increases in CO_2 output only with infected and not with the control leaflets. Yet these leaflets not only had an abundant supply of soluble substrate available, but were using that substrate, as evidenced by its effectiveness in keeping them healthy and in supporting the growth of rust and mildew on them. Although the mobilization of soluble substrates, either from insoluble reserves or from outside sources, may be an important part of the action of toxins from these various parasites, it appears to offer an inadequate basis for explaining the large increases in respiration observed in many cases.

Freedom of enzymes to combine with the substrate.

—It is quite possible that some of the enzymes participating in respiration, though present in sufficient quantity to permit a rapid rate of oxidation, are in the healthy tissue unable to do so because of the blocking of groups essential to their activity. Such blocking might result, for example, from a combination with the enzyme of a substance closely related to its normal substrate or co-enzyme but forming an unreactive complex. Or it could result from an alteration in side groups of the protein, required for activity, but at a different locus from that reserved for either a co-enzyme or a substrate molecule. The appearance in the cell of anything which could effect an un-blocking would, in these circumstances, permit an increase in enzyme activity and, potentially, in the overall rate of respiration. Direct evidence that such effects play a part in eliciting respiratory increases in diseased tissue is lacking. This type of action has received more attention as a basis for the inhibitory action of some toxins, and will be discussed further in that connection.

Availability of co-factors and acceptors.—Recent investigations on the control of metabolism have shown that the availability of inorganic phosphate and of phosphate acceptors may play a critical role in determining rates of oxidation (41, 32). Slow regeneration of ADP, which arises by hydrolysis or by transphosphorylation from ATP, can deprive the system of phosphate acceptors and may at the same time limit the inorganic phosphate available for oxidative phosphorylations, thus slowing down the overall rate of substrate oxidation. A mechanism of this sort was proposed by Johnson (27) as an explanation for the Pasteur effect. The Pasteur effect is the suppression of carbohydrate breakdown in air as compared with the rate of breakdown anaerobically, and has long been recognized as a widespread property of aerobic cells. It is further known that the Pasteur reaction is inhibited by certain substances, with a resulting increase in the rate of aerobic CO_2 production and usually also in O_2 consumption. One of the most extensively studied compounds of this sort is 2,4 dinitrophenol (DNP). Low concentrations of DNP (c. 10^{-5} M) cause marked increases in the respiration of higher plant tissues (47, 39) and of many microorganisms (10). Studies of its mode of action have shown that DNP inhibits the uptake of inorganic phosphate by intact cells (25) and uncouples respiration from the energy-requiring activities of the cell. In cell-free preparations, it also inhibits phosphate uptake (35) and stimulates oxidation. The need for phosphate acceptors in cell-free preparations oxidizing a variety of substrates is eliminated, and the requirement for inorganic phosphate is drastically reduced (32). Although the exact site of action of DNP is not definitely known, it appears likely that it acts upon the energy-rich phosphate substrate formed from oxidative phosphorylation, accelerating the release of inorganic phosphate (26). The consequent rapid turnover of phosphate permits more substrate molecules to be oxidatively phosphorylated, by-passing the rate-limiting phosphate acceptor systems.

Is there evidence that the accelerated respiration elicited by microorganisms and their metabolites is caused by an analogous action? If so, an inhibition of the Pasteur reaction would be expected. Although this question has not been systematically studied, there is evidence that the increased respiration of wheat under the influence of mildew toxins and of fruit under the influence of ethylene is associated with inhibition of the Pasteur effect.

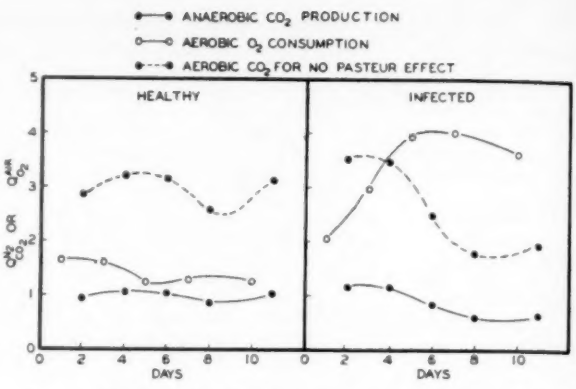

FIG. 1. Loss of the Pasteur reaction in wheat infected with powdery mildew (after Sempio, 1946).

Fig. 1 is derived from Sempio's data (46) on the aerobic and anaerobic metabolism of healthy and mildewed wheat. Assuming an alcoholic fermentation anaerobically, a Pasteur effect is indicated wherever the aerobic CO_2 production is less than 3 times the anaerobic. Carbon dioxide production of healthy wheat leaves is only slightly greater aerobically than anaerobically, indicating that a Pasteur effect is in operation. On the other hand, the aerobic O_2 consumption and CO_2 production of mildewed leaves rises quickly to more than 3 times the level of anaerobic CO_2 production, indicating an abolition of the Pasteur effect. In our laboratory, Miss Weisel has found a slight but definite increase in total phosphorus, most or all of which can be accounted for as inorganic phosphate, without concomitant increases in labile or ester phosphorus. This finding is in accord with the view that the mildew toxins act to accelerate the release of inorganic phosphate and prevent it from being tied up in organic form. Under these conditions, a given level of phosphate acceptors will permit a more rapid rate of oxidative phosphorylations.

Experiments on fruit respiration and ethylene provide still more fully documented evidence. Ethylene is a metabolic product not only of the rot fungus, *Penicillium digitatum*, but also of fruits themselves, and is considered to be responsible for the climacteric rise in respiration (6). If ethylene inhibits the Pasteur reaction, the latter should be operative in pre-climacteric but not in post-climacteric fruit. An example from Kidd and West (29) is reproduced in Fig. 2, and shows exactly this situation. The aerobic CO_2 production of a fruit which had not entered the climacteric is only slightly greater than the anaerobic CO_2 production, while in the post-climacteric fruit, it is about 3 times as great. The rise in aerobic respiration, as indicated

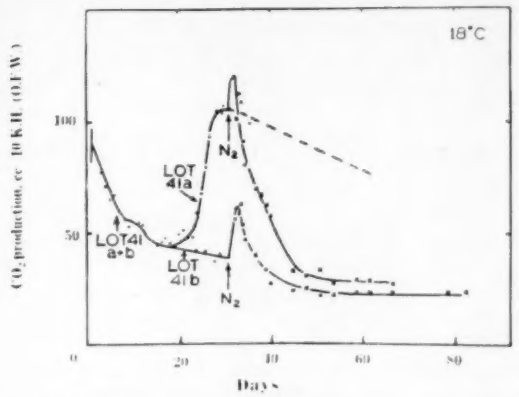

FIG. 2. Relative rates of aerobic and anaerobic CO_2 production before and after the ethylene-induced climacteric in apples. (From Kidd and West, 1945)

by CO_2 output, is almost exactly accounted for by a removal of the aerobic brake on carbohydrate breakdown. Investigations by Biale, Millerd, and Bonner (7), working with the avocado, have further revealed that the respiration of slices of pre-climacteric but not of post-climacteric fruit is increased by DNP, that the oxidation of substrates by mitochondrial preparations requires the addition of a phosphate acceptor (ADP), and that the ADP requirement is reduced by DNP. These findings are strong evidence for the existence in the pre-climacteric fruit of respiratory control exercised by a limited supply of ADP or other phosphate acceptor systems, and for the speeding up of respiration by ethylene as by DNP through eliminating or reducing the requirement for phosphate acceptors. That this action of ethylene is not direct, but is exerted via substances produced under its influence is further indicated by the discovery in ripe but not in pre-climacteric avocados of an agent which stimulates O_2 uptake and suppresses phosphorylation in mitochondrial suspensions.

The similarity between the action of DNP and the physiologically active metabolites of these parasites can be traced still further, at least in the case of those from facultative parasites. The degradation of carbohydrate reserves and the general breakdown of synthetic processes are effected alike by DNP, by ethylene, and by substances at work in the other diseases of storage organs discussed earlier. It is worthy of note, however, that in wheat these latter effects are separated from the initial release of aerobic respiration, and that an active period of synthesis occurs concomitantly with the high respiration rate. This synthetic activity is evident particularly in the accumulation of starch, which in the normal leaf does not appear in mesophyll cells. There may be in this instance a substance with more specific effects than those of DNP and some of the natural toxins of facultative parasites, acting perhaps by increasing the utilization rather than the breakdown of energy-rich phosphate compounds.

Many of the enzymes of respiration require a specific co-enzyme for activity, such as cocarboxylase and co-enzyme I and II. A deficiency of any one of these coenzymes may limit the activity of the corresponding enzyme and hence the overall rate of respiration. If for any reason these cofactors are in short supply, an excess of them or of their precursors in the form of an appropriate growth factor released by a parasite could have an accelerating effect upon respiration. Since most microorganisms synthesize an excess of growth factors, and may release them in considerable quantities (van Lanen, 50), these substances could be instrumental in the increased respiration caused by metabolities of parasitic microorganisms. Hellinga's active principle may be of this nature. If it is pantothenic acid, as he suggested, its effect would depend upon a rapid conversion to coenzyme A by the tuber slices.

This kind of action is different from what is connoted by the term toxic, but so far as the initial result is concerned, it is not readily distinguished from the more deleterious effects of uncoupling respiration from the synthetic processes of the tissue.

EFFECT OF TOXINS ON THE RESPIRATION OF MICROORGANISMS.—So far, attention has been given chiefly to the action of substances produced by microorganisms on the respiration of higher plants. There is abundant evidence that higher plants also produce substances toxic to microorganisms, and even more evidence for the existence and chemical nature of toxins produced by microorganisms and active against others of their kind. These substances include the so-called antibiotics. Generally, the antibiotics which affect respiration and respiratory enzyme systems inhibit rather than stimulate O_2 uptake, but in this respect gramicidin, usnic acid and aureomycin are notable exceptions.

Gramicidin was reported by Dubos, Hotchkiss, and Coburn in 1942 (11) to stimulate the O_2 uptake of *Staphylococcus aureus* exposed to concentrations of 1–40 p.p.m. Hotchkiss (25) later reported that the uptake of inorganic phosphate was also inhibited by gramicidin, and Tepley (48) found that the oxidation of α-ketoglutarate by cell-free preparations was stimulated by gramicidin in the absence of inorganic phosphate. Like DNP, this antibiotic appears to uncouple respiration from synthetic processes, with a resulting arrest of growth. Johnson and co-workers (28) found similar effects of usnic acid ($c.8 \times 10^{-6}$ M) upon oxidation and phosphate uptake in the presence of several of the Kreb's cycle intermediates. The concentrations which inhibited phosphate uptake in homogenates were very similar to those inhibiting bacterial growth. Aureomycin also inhibits phosphate uptake in homogenates without depressing O_2 uptake (34), but the concentrations required are rather high (100–200 p.p.m.).

The other antibiotics affecting respiration more or less directly, inhibit rather than stimulate. Where inhibition is involved, it can reasonably be hoped that one or a few enzymes may be found upon which a toxin is acting directly to block its activity. Reference was made above to the reduction in enzyme activity which may be achieved by substances combining with

TABLE 3.—*Some unsaturated ketones and quinones with antibiotic activity*

Compound	Organisms producing
Unsaturated ketones	
aspergillic acid	*Aspergillus flavus*
kojic acid	*Aspergillus* spp.; *Pen. daleoe*
patulin	*Aspergillus* spp.; *Penicillium* spp.
penicillic acid	*Pen.* spp.; *Asp. ochraceus*
dicoumarol	Sweet clover (spoiled)
protoanemonin	*Anemone pulsatilla*
quercetin	Oak and many other plants
Quinones	
citrinin	*Pen. citrinum; Asp.* spp.
fumigatin	*Asp. fumigatus*
spinulosin	*Pen. spinulosus; Asp. fumigatus*
juglone (5 hydroxy 1,4 naphthoquinone)	*Juglans* spp.
2 methoxy 1,4 naphthoquinone	Garden balsam

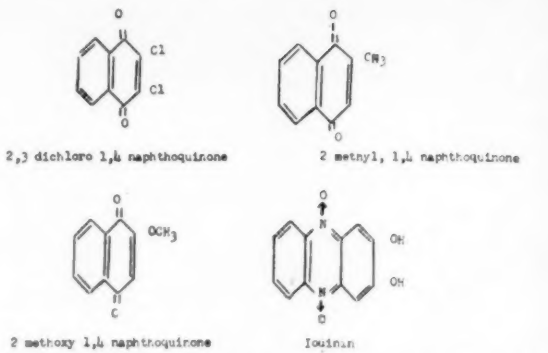

FIG. 3. Analogs of 1,4 naphthoquinone. 2 methyl naphthoquinone will counteract some inhibitions caused by each of the other 3.

an enzyme to block reactive groups. The most extensively studied of these reactive side groups are the -SH groups, on which the activity of many enzymes depends. Several of the enzymes involved in respiration, such as phosphoglyceraldehyde, succinic, malic and alcohol dehydrogenases, and several phosphate-transferring enzymes are -SH enzymes inhibited by -SH inactivating agents. Amongst the agents which inhibit respiration through their action on these enzymes are iodoacetate, p-chloro-mercuribenzoate, unsaturated ketones and quinones. The latter 2 classes include not only some potent fungicidal agents such as 2,3 dichloronaphthoquinone, but also a large group of naturally-occurring antibiotics. Table 3 lists a few representative ketones and quinones which have been identified as antibiotic agents, together with the organism producing each. In a study of the mode of action of unsaturated ketones, Geiger (18) found that several -SH enzymes tested were all inhibited by ketones, which at similar concentrations also inhibited bacterial respiration. Inhibition of respiratory enzymes by patulin, an antibiotic produced by many species of Ascomycetes, was reported by Happold and Waters (21). Most of the enzymes affected were -SH enzymes, but the correlation did not hold completely. In the case of quinones, inhibition of many -SH enzymes has been observed *in vitro* (24), and is considered to be a factor in the antibiotic activity of this type of toxin. Where reactions of this type occur, however, reaction with hydrolytic and other enzymes as well as with the respiratory enzymes is to be expected, and the antibiotic activity may not be mediated primarily through the inhibition of respiration. This is certainly true for antibiotics like penicillin, which reacts with -SH groups but for which inhibition of respiration is not a primary effect and does not occur unless high concentrations are used.

The inhibitory action of substances which react with -SH groups of enzymes can often be reduced or prevented by mercaptans. Particularly effective in this capacity are the naturally-occurring sulfhydryl compounds cysteine and glutathione. These substances will prevent the inhibitory action of the unsaturated ketones (18) and of quinones (16), and in general of any agent which reacts to form a readily reversible complex with the -SH groups of an enzyme (5). These substances and others like them are, therefore, potential protective agents for the cell against the action of the sulfhydryl inactivating toxins. Their potential role in the regulation of respiration may be further appreciated when it is realized that the sulfhydryl groups of enzymes may be blocked in the normal cell, either by oxidation to sulfides or by complexing with substances like the unsaturated ketones and quinones. Under these circumstances, an increase in soluble mercaptans could effect an unblocking of respiratory enzymes with a consequent increase in respiratory rate. Such an unblocking by addition of cysteine or glutathione to enzyme preparations is in fact often necessary before appreciable activity can be obtained.

Inhibition by reaction with -SH groups is not the only mechanism which may be involved in the action of toxins like the quinones. A more specific effect was indicated by the experiments of Ball and co-workers (4) who found that 2 hydroxy, 3 alkyl, 1,4 naphthoquinones, which do not react with mercaptans, inhibited the respiration of *Plasmodium* and O_2 uptake by the succinoxidase system, but only slightly affected succinic dehydrogenase. They attributed the greater inhibition of the succinoxidase system to reaction at a point between cytochrome c and b. Foote and coworkers (15) found that inhibition of carboxylase, which is not a sulfhydryl enzyme, was produced by a number of 1,4 naphthoquinones. Schopfer and Grob (44) found that urease was inhibited by some 1,4 naphthoquinones and not by others which should react with sulfhydryl groups. In a study of the action of some naphthoquinones on the growth of fungi, Wooley (54) found that the toxic action of one analog could be counteracted by another less toxic analog. Thus, for a limited range of concentrations, inhibition by 2,3 dichloro 1,4 naphthoquinone was counteracted competitively by its analog, 2 methyl 1,4 naphthoquinone (Fig. 3). The latter will also overcome the toxic action of 2 methoxy 1,4 naphthoquinone, an anti-

biotic from garden balsam, and of iodinin, a nitrogen analog of the naphthoquinones. This raises the interesting possibility that the toxic action of some of the naphthoquinones may depend upon a successful competition with a chemical analog for a critical position on an enzyme whose activity depends upon the properties of the particular analog combined with it. Particularly in considering the nature of resistance and susceptibility to obligate parasites, the possibility of an interaction of this type between substances from the host and the parasite should not be overlooked. Such an interaction could provide either inhibition or release of inhibition, depending upon the direction of movement between the 2 partners of inhibitory substances and their counteracting analogs. Even normal metabolites may be involved in such interaction, as shown by Williams' report (52) of the inhibition of malic dehydrogenase by ADP and ATP and its counteraction by DPN.

The mode of action of 2 other antibiotics is of particular interest because of their specificity and the good correlation which has been established between the concentrations which inhibit growth and those which inhibit activity of a respiratory enzyme system. These are streptomycin and antimycin A. Streptomycin was observed by Geiger (17) to inhibit the oxidation of certain amino acids which normally followed fumarate oxidation. Umbreit (49) obtained evidence that streptomycin prevents the formation from fumarate of an intermediate required in the oxidation of threonine, and concluded on the basis of further studies that the reaction inhibited was a condensation between oxalacetate and pyruvate. The concentrations of streptomycin which inhibit this reaction are similar to those needed for inhibition of growing cells, and those streptomycin derivatives (dehydrostreptomycin and mannosidostreptomycin) which are antibiotic also inhibit this reaction. Other reactions were found to be insensitive to streptomycin.

Antimycin A, an antibiotic effective against many fungi (33), is one of the most potent antibiotics known, completely inhibiting the growth of yeast at 1.5×10^{-8} M. (1). It inhibits O_2 uptake by yeast and stimulates CO_2 production aerobically but not anaerobically (and thus inhibits the Pasteur reaction). Studies of the site of action have shown that the succinoxidase system is inhibited by concentrations of the same low order as those inhibiting growth (1), and that this action occurs at a point in the succinoxidase system between cytochrome c and b (42). This is the same locus to which Ball and co-workers ascribed the action of the substituted naphthoquinones. The probability that the component affected is identical with Slater's factor was suggested by Potter and co-workers.

SUMMARY AND CONCLUSIONS

In the foregoing discussion, an attempt has been made not to cover all phases of this subject, but rather to bring out a few of the more striking or interesting aspects of the effects of toxins on respiration, particularly those which might be applicable to the relations between a host and its parasite. One of the striking facts which emerges is the widespread occurrence of respiratory increases as an early effect of the action of toxins on higher plants. The limited evidence that this results from an uncoupling of respiration from energy-requiring processes through an action on oxidative phosphorylations is given greater weight by the finding that this effect is also produced by several purified antibiotics—gramicidin, usnic acid, and aureomycin. From the point of view of a parasite bent on obtaining nourishment from its host, this may accomplish a very useful purpose. The normal mature host cells are geared to a conservative but efficient economy, with their metabolism regulated to prevent an undue expenditure of reserves yet providing enough energy and materials for maintenance of their protoplasm. With the abolition of the regulatory mechanism, the reserves of the host and intermediates formed from them become rapidly available to the parasite. Incidental to this, the utilization of energy by the host cells is impaired, and their death follows from lack of available energy, undoubtedly aided by other less subtle factors in the case of the destructive parasites. With the obligate parasites, there is some reason to believe than an acceleration of degradative processes is not accompanied by the complete uncoupling of synthetic processes in the host cells, so that these cells are able for some time to maintain themselves and continue to turn out an abundance of metabolic intermediates for the parasite—provided the basic materials are replenished as needed from an outside source, such as a solution of carbohydrate, or other healthy leaves of the same plant.

A second point which deserves emphasis is the generally inhibitory effect upon respiration and respiratory enzymes of those toxins which are active against microorganisms. The function of these substances—if they have a function—is to defend the organism that produces them against an invader or a competitor, an aim which can be nicely accomplished by a straightforward inhibition of some critical enzyme of the microorganism. Whether the action of a toxin is by inhibition or by stimulation, however, the eventual understanding of this action will depend upon a knowledge of how and where enzyme systems are affected. In the pursuit of this knowledge, there may well emerge new and interesting facts and ideas, not only about the mode of interaction of organisms, but also about the nature and control of enzyme action in the normal cell.

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ON THE NATURE AND ROLE OF VIVOTOXINS IN PLANT DISEASE [1]

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In certain plant diseases, pathologists have for a number of years debated whether or not a portion of the syndrome may be caused by toxic substances produced in the host by the pathogen. Such substances have been called toxins. In some diseases toxins are thought to cause a more rapid and extensive invasion by the pathogen than would be the case in the absence of a toxin; it has even been suggested that some parasites would be unsuccessful if the toxin did not kill cells in advance of the fungus and permit it to establish itself continually on dead or dying cells and produce more toxin (17, 32). In other diseases toxins are thought to be translocated from the center of infection and produce conspicuous disease symptoms which are thus of secondary origin, but of primary consequence to the infected host. In either case a mechanism for disease control is suggested; by rendering the toxin inactive, one may cause the fungus to fail as a pathogen (31, 32). Consequently, a knowledge of the identity and chemistry of a toxin will make possible the prediction of compounds which will inactivate it and bring about disease control.

Unfortunately, toxic compounds, for example, components of culture filtrates, have been too quickly ascribed a role in plant diseases. The writers take a conservative attitude toward the role of these toxic compounds in disease production and propose to discriminate between them and vivotoxins, toxic compounds which produce a portion of the symptoms of disease in the naturally infected plant. Criteria for establishing the vivotoxic nature of a compound follow. Several examples of these naturally occurring pathogenic compounds can be cited. Finally mechanisms by which vivotoxins can cause plant disease are suggested.

The word "toxin" has been used with so many different meanings that its precise definition has become obscured. In the dictionary the term toxin is limited to the medical usage and includes only certain specific nitrogenous compounds, usually produced by bacteria, which have a high potency, e.g., botulinus toxin. In plant pathology compounds produced by microorganisms which are toxic to higher plants have been called toxins. Thus Gaumann (18) has said that such toxic compounds as the enniatins, javanacin and lycomarasmin are "wilting toxins." Some have considered that such compounds are responsible for symptom production in plant diseases, when, in point of fact, this has not been demonstrated. In other cases the word toxin has been limited to compounds thought to have a role in the pathology of infected plants. Loose usage of the word "toxin" has led to confusion in the study of disease causes.

To clarify this situation a new term is suggested. A *vivotoxin* is defined as a substance produced in the infected host by the pathogen and/or its host, which functions in the production of disease, but is not itself the initial inciting agent of disease. Vivo is used as a prefix to associate the term with *in vivo*. In contrast a *toxin* is any compound produced by a microorganism which is toxic to plants. Toxins isolated from culture filtrates are not regarded as vivotoxins unless they have also been shown to be present in the naturally infected host and functional in disease production.

CRITERIA FOR VIVOTOXINS.—Conservative criteria are necessary for establishing the existence of a vivotoxin, as is evident from the many instances where toxins have been ascribed a role in disease production on inadequate grounds. A vivotoxin is a disease-producing entity and therefore a pathogenic agent. Koch's postulates need but slight modification to be made applicable to proving the pathogenicity of vivotoxins. Reproducible separation from the sick plant, purification, and reproduction of at least a portion of the disease syndrome by placing the toxin in a healthy plant should be the minimum conditions satisfied before vivotoxicity is said to exist. Because the vivotoxin may[2] not be reproduced in the host in the absence of the pathogen, one cannot require that it be reisolated from the plant into which it was introduced.

If, in the future, it is found that 1 vivotoxin is active in a number of diseases, and its mode of action is well understood, it may then be possible to identify this vivo-

[1] An invitational paper presented at the Symposium on Physiology of Parasitism at the 44th Annual Meeting of The American Phytopathological Society in Ithaca, N. Y., September 9, 1952.

[2] Hall (27) has concluded that ethylene, produced by injured plants, also injures them, thus stimulating more ethylene production. This is an example of a vivotoxin which leads to the production of more vivotoxin. Viruses are not vivotoxins because they are initial inciting agents of disease. However, a virus in the infected plant may cause the host to produce a vivotoxin which causes a portion of the syndrome. In this instance the vivotoxin and the virus are obviously separate entities.

toxin in a new disease by its characteristics and without isolating it. For the present, however, it appears that Koch's postulates as modified are not only sufficient but also necessary. The identification by Braun (3) of the vivotoxin responsible for the halo symptoms about centers of infection in wildfire of tobacco is an excellent example of proof of vivotoxicity by the 3 steps given above.

That a vivotoxin is involved can be demonstrated by the first and third steps only. But just as it is usually necessary to know the identity of a parasite to establish it as a cause of disease, so it is also necessary to purify and identify a vivotoxin to prove its complicity. When this has been done a rational program of chemotherapy can be undertaken, involving a destruction of the vivotoxic mechanism.

Isolation of a toxin from culture filtrates is only a step in establishing the presence and nature of a vivotoxin and may make easier its identification in the diseased plant. Until the suspected material has been isolated from the sick plant, there is no justification for ascribing vivotoxic properties to it.

Both toxins and vivotoxins are specific metabolic products of the pathogen on a given substrate. Knowing how much an alteration in the nutrient medium can affect the presence or the yield of a specific product of fungus metabolism, e.g., penicillin, we should expect that not all toxins in culture filtrates will be formed in the naturally infected host and that not all vivotoxins will be formed on artificial culture media. Many organic compounds, possible vivotoxin precursors, are present in the infected plant which are not present in synthetic media. The effect of precursor supply upon metabolic product yield has been well demonstrated in studies of penicillin production (2). Therefore, when one studies culture filtrates, he is in danger not only of not finding a vivotoxin but also of mistaking a toxic component of the culture filtrate for a vivotoxin.

Resemblance of disease symptoms to those produced in the noninfected host by a toxin is likewise apt to be misleading. Plants have a limited number of ways of expressing symptoms and many chemically unrelated compounds can produce similar symptoms. Similarity of symptoms in such instances can be useful in inferring a mode of action upon host cells, but by itself offers little more information. The symptoms produced on tomato by cucumber mosaic virus closely resemble those produced by spraying tomato foliage with certain growth regulators. This may be evidence for the similarity of action of the virus and growth regulator, but is certainly not evidence that the virus and the growth regulators are identical or even chemically related entities. Yet in too many instances our conclusion that vivotoxins function in a plant disease has rested on just such arguments.

EXAMPLES OF VIVOTOXINS.—Vivotoxins have been isolated from diseased plants in a few cases. Braun has obtained the wildfire vivotoxin from culture filtrates and from diseased plants. Moreover, he has studied its biochemistry (3) and recently this vivotoxin has been purified (57). In this case, only the chemistry of the active compound remains to be determined, and this is strongly suggested by recent work (3, 57).

Ethylene has been shown to be a cause of defoliation in plant diseases (53) and subsequent studies on the role of the auxin-ethylene balance in controlling defoliation (28) have made this the first instance in which the complete picture is at hand. In both these cases the mode of toxic action is sufficiently understood that research can now be undertaken for disease control. These modes of action will be discussed.

In contrast are the cases where vivotoxins are known to be involved but their nature and mode of action are as yet undisclosed. Because the nature of disease resistance may be involved in some of these cases, they are discussed briefly.

From plants infected with *Fusarium oxysporum* f. *lycopersici*, Gottlieb isolated a vivotoxin, displaced from vascular elements of the stem by centrifuging, which was stable to oxygen, caused reversible wilting of cuttings, was heat stable at 100°, and increased cell permeability (25, 26). The reversible nature of this vivotoxic effect indicates that Gottlieb was not dealing with lycomarasmin.

Of unique interest is the vivotoxin associated with Victoria blight of oats. In 1946 Meehan and Murphy described a new disease, caused by *Helminthosporium victoriae* (37). This pathogen specifically attacks Victoria oats and derivatives which have high resistance to crown rust. In the following year, they described a toxin from cultures of *H. victoriae* which caused injury of only those oat varieties susceptible to Victoria blight and resistant to crown rust (38). Subsequently Litzenberger (34) partially demonstrated the vivotoxic nature of this substance by isolating a similar toxin from blighted Victoria oats and from rusted Bond oats (*Helminthosporium* resistant) as well as from *H. victoriae* cultures. Because all derivatives of Victoria oats were both susceptible to *Helminthosporium* and resistant to *Puccinia*, he concluded that resistance to the obligately parasitic *Puccinia* and susceptibility to the facultatively parasitic *Helminthosporium* were controlled by a single gene. Thus we have a striking example: what is a vivotoxin in one disease appears to cause the plant to be resistant to another disease.

The chemical nature of this vivotoxin is not yet known, nor is the mode of action. A knowledge of these may be expected to offer suggestions as to the nature of disease resistance and possibly to methods of disease control.

In a few instances, toxins from culture filtrates have been purified, even chemically characterized. Some of them produce symptoms when applied to plants which are said to resemble the symptoms of diseased plants. With such compounds as lycomarasmin (20, 43, 55) and alternaric acid (4, 6, 44) recent studies (5, 14, 52) have indicated that although the toxins cause symptoms similar to tomato wilt or early blight, they probably do

not function in production of these 2 diseases. Of course, isolation of phytotoxic quantities from the diseased plant is all that is needed to prove vivotoxicity in the case of these toxins. Because their chemical properties are known, isolation of these compounds from diseased plants need not be empirical.

The problem of attempting to demonstrate the vivotoxic nature of a toxin is exemplified by lycomarasmin. Lycomarasmin has not been isolated from diseased plants but can be isolated from quiet cultures, grown on Richard's solution for 2–4 months. Analyses of culture filtrates during the course of such a fermentation reveal that after 3 weeks glucose is depleted, that nitrates are utilized during this period and then ammonium is consumed. During this period, the pH rises from an initial value of 4 to a value of 8 by the end of the fifth week. After glucose is depleted, polysaccharides increase slowly, rising as high as 1 mg/ml, a concentration on the threshhold of toxicity. Maximum mycelial weight is attained within 5 weeks, and thereafter it decreases as lysis occurs. By the end of the eighth week, the lysis process is well under way. At this time lycomarasmin appears (14). Lycomarasmin has been isolated from cultures of 8 weeks and older by several workers (43, 46, 55).

Lycomarasmin characteristically produces a shock effect upon plants (20). This effect has not been observed in diseased plants in which the course of transpiration has been followed (35, 36, 46). Scheffer (46) has estimated that lycomarasmin production by cultures of *Fusarium* is less by orders of magnitude than would be required to cause wilting in tomatoes within the time that inoculated plants succumb to disease. His estimates are on the lenient side because lycomarasmin in aqueous solution loses its activity through decomposition within a few hours (15, 43).

As is well known, Fusarium wilt causes wilting of leaves and discoloration of the vascular system. In experiments where inoculated plants, treated with many chemotherapeutants (12), with maleic hydrazide (50), and with X rays (51) both of which increase severity of disease, the correlation between the severity of disease as measured by grading leaf symptoms and by grading vascular discoloration has been exceedingly high. Lycomarasmin does not produce vascular discoloration (19, 46). These results suggest that there is a common cause for vascular discoloration and wilting of leaves or that there is a cause and effect relationship between vascular occlusion and foliage symptoms. That the latter is the more probable is suggested by the work of Scheffer (46) discussed below.

SOME PROBABLE MECHANISMS OF ACTION BY VIVOTOXINS.—Vivotoxins differ from other poisons only in the conditions under which they are produced. Their modes of action, however, probably do not differ from those of other poisons. Accordingly the subject is vast and the literature of pharmacology and chemotherapy is helpful in suggesting possible mechanisms. (56, 58). In the following discussion, however, illustrations have been chosen which are related to the field of plant pathology, although many of these mechanisms have not yet been demonstrated with proven vivotoxins.

Physical mechanisms.—A) *Altered permeability of the host cell.*—The ability of toxins from culture filtrates to increase the permeability of the host cell has been measured many times (18, 20, 21, 25, 26, 40, 47, 48). That this occurs is undeniable, but whether a change in cell permeability in response to the application of a toxin is cause or effect is debatable. Any compound which seriously injures the cell will alter its permeability.

There is confusion concerning the role played by altered permeability in the toxic action of lycomarasmin. Lycomarasmin is said to increase the permeability of cells (18). However, the only published data on this effect show that when beet slices are immersed in lycomarasmin solutions they do not lose anthocyanin to the ambient solution (21). The effect of lycomarasmin in stopping protoplasmic streaming of cells of *Tradescantia* (18) but not *Elodea* (21) could be secondary to other cellular injury. The toxic action of lycomarasmin has also been attributed to its coagulating effect on protoplasm—an effect noted from 2–3½ hours after protoplasmic streaming has ceased in *Tradescantia* (18). Coagulation, therefore, could be a final, observable result of earlier toxic action.

In view of these facts it is difficult to ascribe to any known toxin the ability to alter permeability as a primary mode of toxic action. Such compounds may eventually be found.

B) *Vascular occlusion.*—When polysaccharides enter the vascular elements of tomato cuttings, they cause wilting to occur (29). Polysaccharides have been isolated from cultures of a variety of bacterial plant pathogens (16, 30) and from wilt-producing fungi (13, 14, 49). In vascular wilt diseases where the pathogen grows in vessels, it seems quite possible that polysaccharides can be liberated into the vascular system in sufficient amounts to cause wilting. In certain cases polysaccharides and other hydrophilic compounds are sufficiently abundant in diseased tracheal elements to be used as diagnostic symptoms for the disease; when this occurs, the polysaccharides are almost surely a cause of wilting; e.g., bacterial wilt of cucumber and Stewart's wilt of corn.

Four lines of evidence indicate that the effect of polysaccharides in conductive tissues is entirely of physical nature, water shortage being brought on by blocked capillaries which are the size of the polysaccharide molecule. Other polymers produce similar effects on cuttings when their molecular dimensions approach those of polysaccharides (29). Large amounts of polysaccharides are required to produce wilting. Thus the sensitivity of tomato cuttings is of the order of 1700 mg inulin per kg. fresh weight of tomato tissue (18, 22). Further, polysaccharides disrupt the water relations of a wider range of plants than any other toxic substance isolated from microorganisms. In contrast with cellular toxins, it is known that

inulin does not affect the permeability of beet slices because no anthocyanin is lost to the ambient solution. Furthermore, inulin (10^{-3} M) has no effect on the rate of protoplasmic streaming in *Elodea* cells (18).

Chemical mechanisms.—A) *Enzymes as vivotoxins.*—In some vascular wilt diseases there is a high correlation between vascular discoloration and occlusion of vessel elements. Examples known from a demonstrated reduction in water movement are Dutch elm disease (59) and Fusarium wilt of tomato (35, 36, 46). Impeded movement of solutes in the transpiration stream has also been demonstrated by use of radioisotopes for oak wilt (1) and probably for Fusarium wilt (50, 51).

The mechanism by which the translocative dysfunction and the ensuing vascular discoloration is brought on is of more than passing interest, because it appears to be operative in a number of diseases. When the mechanism is known, the chemotherapy of these economically important diseases can be guided rather than empirical.

Before discoloration appears in *Fusarium*-infected vessels, they contain a viscous, transparent occluding substance which is removed from microscopic sections during fixation and staining (35, 36). Recently Scheffer (46) demonstrated occlusion of discolored stem and petiole segments, and has suggested that the principal wilting of tomato foliage results from this effect. Because the occluding material frequently fills a vessel but the mycelium can rarely be found as an occluding agent, a vivotoxin must be operative. We can infer a mechanism for a vivotoxin which behaves in this manner. A hydrolytic enzyme, produced by the pathogen and attacking lignin or other constituent of vessels or adjacent cells, might yield colored and viscous substances which would occlude the conductive elements of stem and petiole.

In an attempt to obtain a toxin producing the occlusion and discoloration of conductive elements Scheffer macerated young mycelia of *Fusarium* and suspended the macerate in water for 24 hours. The resulting filtrate contained a substance which would produce both the discoloration and occlusion of tomato cuttings. The active portion of the filtrate is heat labile and possibly an enzyme (46). Concurrently, Gaumann (19) has isolated a toxin with similar properties which may or may not be the same as that Scheffer is concerned with. In any case the vivotoxic nature of the substance remains to be demonstrated.

Secretion by a pathogen of an enzyme which then attacks the host in advance of mycelial growth is the mechanism for vivotoxic action that is best known. The classical studies which led to isolation of pectic enzymes from cultures of plant pathogens producing soft rots (7, 11, 39), need no more than passing mention. Without doubt this mechanism is common to many other enzymes secreted by plant pathogens.

B) *Competitive inhibition.*—A toxin is said to be a competitive inhibitor if it competes with a substrate for specific groups of an essential enzyme so that the consequent decrease in activity of the enzyme depends upon the relative concentration of both substrate and inhibitor (33). Two known vivotoxins are competitive inhibitors.

1) *Ethylene.*—Ethylene causes epinasty, defoliation, and fruit drop—all common symptoms of disease. The effect of ethylene can be inhibited by indole acetic acid and the molecular ratio of ethylene to indole acetic acid is probably a constant for a constant response (28).

As a vivotoxin ethylene has 2 noteworthy characteristics: a) it can be formed by either the infected host or by the pathogen itself, and b) it is formed somewhat by the normal host, but its production is accelerated to a toxic level when the plant is injured or attacked by a pathogen. Citrus fruits with stem end rot have been shown to liberate more ethylene than normally ripening fruits (41). Further, *Penicillium digitatum*, the causal organism of stem end rot, is the only plant pathogen so far found to produce ethylene (53). However, rose leaves attacked by black spot and cherry leaves attacked by shot hole disease produce substantially more ethylene than corresponding healthy leaves (53). Apparently ethylene production is a result of injury to host tissue by the pathogen and not a product of the pathogen itself, and the ethylene is responsible for the defoliation so characteristic of these diseases (53). An enzyme which will attack pectin and a number of carbohydrates to produce ethylene has been isolated from *P. digitatum* and from apple juice (27). Presumably this same enzyme is active in the injured plant.

Hall (28) has recently substantiated a hypothesis concerning defoliation by ethylene. In the normal leaf there is an auxin gradient across the abscission layer, a gradient which disappears when the leaf becomes old or is injured. In the young, healthy leaf, the gradient is maintained by continual auxin synthesis. Maturation of the cells in the abscission layer and defoliation are postulated to depend upon the relative auxin and ethylene concentrations there. This was tested by applying ethylene chlorhydrin and indole acetic acid to debladed petioles or naphthalene acetic acid sprays followed by ethylene gas to entire plants. In all instances the hypothesis was verified.

The demonstration of ethylene evolution by pathogens and by infected tissues (28, 53), the observation that pathogenic fungi produce growth-regulating substances (42), and the chemotherapeutic activity[3] of many growth regulators (10) all suggest that studies of the role of the auxin-ethylene balance in disease will be most profitable.

2) *Wildfire toxin.* From Braun's careful work on the biochemical behavior of the wildfire toxin (3), we have

[3] The chemotherapeutic activity of non-formative derivatives of phenoxyacetic acid was demonstrated by Crowdy and Wain (8, 9). The design of their assay (9) precludes the possibility that the effects they observed depended upon an altered auxin-ethylene balance affecting defoliation.

another clear example of competitive inhibition by a vivotoxin. Wildfire of tobacco, caused by *Pseudomonas tabaci*, characteristically produces lesions which are surrounded by a chlorotic halo. The pathogen produces a toxin, both in culture and in the host, which produces a similar halo on a variety of plants (3). Inhibition of the growth of *Chlorella vulgaris* on synthetic media by the toxin is overcome when l-methionine is added, but the toxin is not inactivated. Similarly toxic filtrates to which methionine was added failed to produce a chlorotic halo on tobacco leaves. However, when methionine was applied to a toxin-produced halo, the halo remained (3). Because the addition of l-methionine to the toxin in a constant ratio appeared most effective in overcoming the toxic effect, Braun reasoned that methionine utilization is prevented by the toxin, an effect overcome when an abundance of methionine is supplied. When the known methionine antagonist, methionine sulfoxime, was applied to tobacco leaves, it produced halos indistinguishable from those produced by the wildfire vivotoxin (3).

3) *Reaction with sulfhydryl groups.*—A number of enzymes depend upon reactive sulfhydryl groups for their activity, e.g., glyoxalase. Cysteine and glutathione are both essential metabolites, and toxins which are specifically reactive for these groups could inactivate the enzymes. If, as in glyoxalase (45), the sulfhydryl group is the site of substrate attachment, inhibitors would be competitive. Otherwise, the inhibitors would inactivate both enzyme and enzyme-substrate complex and be non-competitive.

Although not yet shown to be a vivotoxin, the antibiotic patulin (clavacin) is an example of a sulfhydryl inactivating toxin. Patulin is highly toxic to plant cells and tomato cuttings (18, 21, 40). It is inactivated as an antibiotic against bacteria by compounds containing free sulfhydryl groups (24). Miescher (40) found that plants treated with patulin contain about half the normal amount of sulfhydryl compounds. Further, when plants in which the sulfhydryl groups have been oxidized by placing the plants in the dark are treated with patulin, the toxic effect is greater than when plants held in the light are treated.

C) *Non-competitive inhibition.*—If a toxin inactivates an enzyme by combination with groups not concerned with substrate, it is said to be a non-competitive inhibitor. Unlike competitive inhibitors which cause a response depending upon the ratio of inhibitor and substrate, non-competitive inhibitors cause a response dependent only on concentration of inhibitor (33).

1) *Lycomarasmin.*—Although not yet shown to be a vivotoxin, lycomarasmin will be used as an illustration of a non-competitive inhibitor. Strepogenin, a growth factor for *Lactobacillus casei*, is inhibited by lycomarasmin when added to cultures of *L. casei* (54). Strepogenin also counteracts the toxic effects of lycomarasmin on tomato cuttings (23, 54). The inhibition of strepogenin by lycomarasmin has been shown to be independent of strepogenin concentration (56). Although the structure of strepogenin is not yet known, there is reasonable evidence that it is similar to that of lycomarasmin (54, 55, 56). Thus lycomarasmin is a peptide derivative of α hydroxyalanine, glycine and asparagine, whereas strepogenin activity is shown by seryl glycyl glutamic acid (serine being β hydroxyalanine) (56).

Lycomarasmin possesses a unique characteristic which may be related to its role as a non-competitive inhibitor. It has been shown to form chelate complexes with metals (52). With iron, but not with other metals, lycomarasmin is about 10 times as toxic as when it enters the plant in pure form (18). As lycomarasmin loses toxicity through heating, it also loses iron-chelating ability (15). Its unique potentiation by iron and the relation of toxicity to chelating ability suggests that it acts as the iron complex and not by removing iron from enzyme systems.

Recent studies have indicated that chelating of iron by lycomarasmin probably involves the amide N and the carboxy groups of the asparagine moiety (15). Strepogenin, however, with a glutamic acid or glutamine moiety occupying an analogous position in its structure, can be predicted to form chelate complexes of lower stability if they are formed at all. These relations are probably involved in the inhibition of lycomarasmin toxicity by strepogenin.

SUMMARY

A toxin is any compound produced by a microorganism which is toxic to plants. A vivotoxin is defined as a substance produced in the infected host by the pathogen and/or its host, and which functions in the production of disease but is not itself the initial inciting agent of disease. Koch's postulates are modified to establish criteria for proving the pathogenicity of vivotoxins. These criteria are a) reproducible separation from the sick plant, b) purification, and c) reproduction of at least a portion of the disease syndrome when the toxin is placed in a healthy plant. Vivotoxicity has been established in a few instances which are discussed.

The problem of demonstrating vivotoxicity of a toxin is discussed in terms of lycomarasmin. Evidence is presented which indicates that if lycomarasmin is functional, it probably does not play a primary role in the pathology of Fusarium wilt of tomato.

Vivotoxins can act by any mechanism by which other poisons affect cells. Toxins can act physically by altering cellular permeability and by occluding the conducting elements of the vascular system. Polysaccharides commonly are a cause of the latter. The modes of chemical action are more varied. Extracellular enzymes are well established as vivotoxins, and it is suggested that the vascular discoloration characteristic of certain wilt diseases results from enzymic attack of vascular tissue.

Competitive inhibitors include ethylene, an example of a vivotoxin which the host can produce in response

to injury by the pathogen. Competitive inhibition of methionine utilization has been established as a *modus operandi* for the wildfire vivotoxin.

Inhibition by a vivotoxin might depend upon a specific affinity for cations or for sulfhydryl groups. Patulin is cited as an example of the latter. Non-competitive inhibition is illustrated by lycomarasmin, which acts as an antimetabolite for the growth factor strepogenin.

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DETECTION OF MICROBIAL TOXINS BY THE USE OF RADIOISOTOPES[1]

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Evidence regarding the role of microbial toxins in plant disease has been derived chiefly from observations of disease symptoms in plant tissues not occupied by pathogens and from studies of host reaction to toxic agents produced by plant pathogens in artificial culture. Such evidence, while in many cases highly suggestive, has not provided conclusive proof that disease symptoms in plants result from the direct action of toxins produced by plant pathogens. Destruction of host cells in advance of their penetration by the pathogen might be attributed to the action of toxic substances released by the host itself rather than to toxins produced by the pathogen. Also, the possibility that toxins produced by pathogens in artificial culture may not be produced or may not be active in host tissues must be considered. The lack of specificity of toxins produced in artificial culture (8) and the fact that their production may be affected by the composition of the medium (2) and often is not correlated with pathogenicity (5, 7), emphasizes the need for caution in extrapolation of results obtained *in vitro* to the host-parasite relationship. In a few cases, toxic substances have been isolated directly from infected plants. In one such case, however, a toxic substance was also obtained from healthy plants (11), and in another the author pointed out that it was not possible to determine whether the toxin was a metabolic product of the pathogen or a reaction product of the host (6). Gäumann (5) has pointed out the circumstantial nature of the evidence which indicates that specific microbial toxins are responsible for pathological wilting in plants and has stressed the need for methods by which toxins produced by pathogens within host tissues can be detected and distinguished from host reaction products. The possibility that microbial toxins labeled by the use of radioisotopes might provide a tool for the development of such methods has been suggested (16, 18).

PROBLEMS INVOLVED IN THE PRODUCTION OF LABELED TOXINS.—The successful biosynthesis of many labeled products of microbial metabolism (4, 9) indicates that labeled toxins can be produced *in vitro* without difficulty by the addition of appropriate radioactive compounds to the culture medium. Labeled toxins produced *in vitro* should provide valuable tools for investigations concerning the distribution and fate of such toxins when introduced into plants, particularly toxins such as lycomarasamine or alternaric acid for which procedures for isolation and purification have been developed (1, 13, 14). The use of labeled toxins produced *in vitro* will, however, yield only indirect evidence regarding the production and action of microbial toxins within host tissues. Resolution of these problems seems dependent upon the development of methods for the production of labeled toxins *in vivo*.

The use of plant pathogens tagged (rendered radioactive) by a period of growth on a medium containing an appropriate radioisotope has been suggested as an approach to the problem of the production of labeled toxins *in vivo* (16, 18, 19). Theoretically, toxins produced by tagged plant pathogens should be radioactive and inoculation of plants with tagged pathogens should result in the elaboration of labeled toxins within the host. Although methods for tagging plant pathogenic fungi and bacteria have been described (16, 17, 18, 19), their use for the production of labeled toxins *in vivo* requires the solution of several technical problems. One of the most difficult of these is the problem of rendering the pathogen sufficiently radioactive to permit detection of radioactivity in its metabolic products without causing excessive damage to the tagged organism. Since only a small fraction of the radioactivity of a tagged pathogen would be incorporated into its metabolic products, it seems reasonable to assume that the initial concentration of radioactivity in the tagged pathogen must be 10^3 to 10^4 times greater than the minimum required for detection with available assay methods. The determination of the level of radioactivity that plant pathogens will tolerate without loss of pathogenicity or other vital functions is, therefore, a problem of major importance.

The severity of damage caused by ionizing radiations depends upon the quantity, type, energy, and distribution of ionizing particles within biological material (10). Since different radioisotopes emit different types of ionizing particles having various energies, an estimate of the level of radioactivity which plant pathogens will tolerate can be made only for specific radioisotopes. This leads to a consideration of the radioisotopes best suited to the production of labeled toxins. Obviously, only radioisotopes of elements which occur both in plant pathogens and in the toxins they produce can be utilized. Chemically identified toxins produced by plant pathogens include peptides, quinones, glucosans, and organic acids (1, 5, 8, 13). These substances have only 3 elements in common; carbon, hydrogen, and oxygen. These are also the elements which occur most abundantly in plant pathogens. Two radioisotopes of carbon (C^{11} and C^{14}), 1 of hydrogen (H^3 or tritium), and none of oxygen are available. The short half-life of C^{11} (20 minutes) makes this isotope unsuitable, hence, C^{14} and H^3 appear to be the most generally useful radioisotopes for the production of labeled toxins. These isotopes are weak beta emitters and have half-lives more than adequate for the uses under consideration. Carbon^{14} has been used extensively in biological research and procedures for its use and assay are well established (9). Less information is available for tritium (H^3) and the large mass ratio of tritium (H^3)

[1] An invitational paper presented at the Symposium on Physiology of Parasitism at the 44th Annual Meeting of The American Phytopathological Society in Ithaca, N. Y., September 9, 1952.

to protium (H^1) may be expected to lead to isotope effects if tritium is used as a tracer for hydrogen (9). It has been suggested, however, that tritium may be used as an auxiliary tracer for carbon (9, 11) and certain characteristics of this isotope indicate that it may prove very useful for tagging plant pathogens.

Data concerning the damage caused to microorganisms by the beta particles emitted by C^{14} and H^3 are limited. Wheeler (19) reported that 5 species of plant pathogenic fungi grew satisfactorily on a medium containing C^{14} labeled sucrose and having an activity of 30 microcuries/ml. Fungal spores, tagged by a 5–7 day growth period on this medium, exhibited no lethal or mutagenic effects and showed no loss of pathogenicity. Survival data obtained from longer periods of exposure to this medium indicated that, with C^{14}, activities several times greater than 30 microcuries/ml. might be used for tagging plant pathogens. Fitzgerald, et al (3) grew yeast for a period of 4 days in a medium containing tritium and having an activity of approximately 1 millicurie/ml. While the yeast cells were capable of budding after this exposure, these investigators did not state whether changes in the growth or physiology of the organisms were produced. In investigations, the results of which have not been published,[2] media containing 100 microcuries of C^{14}/ml. and media containing 1 millicurie of H^3/ml. have been used for tagging plant pathogens. Although growth was retarded and approximately 25 per cent of the cells were rendered nonviable, no loss of pathogenicity of the surviving organisms was observed. On the basis of these data, it seems probable that tagged organisms can be used for inoculation if 50 per cent of the cells are viable. If this is assumed to be the case, rough estimates of maximum possible dosages can be calculated from survival data obtained by treating various microorganisms with X and beta rays (10, 15). These data indicate that approximately 3000 rep. (roentgen equivalent physical) are required to kill 50 per cent of bacterial cells or fungal spores suspended in aqueous medium. If a 5-day period is used for tagging purposes, the maximum daily dose would be 600 rep. This can be converted to microcuries/gm. by following formula (9), $C = 600/(60\ E_{av})$ where C is the maximum permissible concentration in microcuries/gm. and E_{av} the average energy in mev. (million electron volts) of the emitted beta particles. Thus, with C^{14} having an average energy of 0.052 mev., the maximum permissible concentration in the tagged pathogen is approximately 190 microcuries/gm. and with H^3 (average energy 0.006 mev.), 1700 microcuries/gm. An inoculum consisting of 1 milligram of the tagged pathogen would have a total activity of approximately 4.2×10^5 disintegrations/min. if C^{14} were used or 3.8×10^6 disintegrations/min. if H^3 were used. Toxins produced in plants inoculated with pathogens tagged with C^{14} could be detected if as much as 1/1000 of the activity in the tagged inoculum was released in form of toxin. With tritium, detection would be possible if only 1/10,000 of the initial activity in the tagged pathogen occurred in the toxic product.

The estimates given above indicate that labeled toxins can be produced *in vivo* by infecting plants with tagged pathogens. Preliminary studies (unpublished) of *in vitro* toxin production by tagged cultures of *Helminthosporium victoriae* offer some indirect evidence in support of this view. Approximately 10^7 tagged spores from a culture grown for 5 days on a medium containing 100 microcuries/ml. of C^{14} were washed and transferred to a flask containing 10 ml. of nonradioactive liquid medium. Two ml. aliquots were taken daily, filtered to remove the fungus, and the filtrates tested for radioactivity and toxicity. No radioactivity or toxicity could be detected in filtrates taken after 24 hours, but both were demonstrated in filtrates taken after 48 hours. Subsequent samples revealed a close positive correlation between radioactivity and toxicity of the filtrates and both increased logarithmically from the second through the fifth day. The toxicity of filtrates from nontagged control cultures did not differ significantly from that of filtrates from the radioactive cultures. While direct extrapolation of these results to the host-parasite relationship is not possible, they demonstrate that toxic filtrates which contain easily detectible concentrations of radioactivity can be obtained from tagged fungi.

It should not be inferred from the foregoing discussion that maximum levels of radioactivity are desirable in biological tracer research. The use of high levels of radioactivity increases the possibility of effects due to internal radiation which may interfere with the biological processes under investigation. If high levels must be used, precautions should be taken to eliminate or compensate for such effects. It is probable that by using large quantities of inoculum, labeled toxins may be produced in plants with pathogens tagged at levels of radioactivity considerably below the maximum.

Detection of labeled toxins produced in vivo.—A detailed discussion of the problems involved in extraction, purification, and assay of labeled toxins produced *in vivo* is beyond the scope of this paper. In general, the methods used for detection and assay of radioactivity in metabolic products of microorganisms and the fractionation and degradation procedures employed in tracer studies of intermediary metabolism (4, 9), should serve as useful guides for the development of methods for isolating labeled toxins from plants. Two methods, which seem particularly adapted to use with labeled toxins produced by tagged plant pathogens are autoradiography of histological sections and chromatographic analysis of extracts of plants infected with tagged pathogens.

Autoradiography offers an excellent tool for studies of the distribution of radioactivity within tissues. Methods of autoradiographing fungal spores and mycelium tagged with C^{14} and yeast cells tagged with H^3 have

[2] Unpublished and published studies reported in this paper were carried out under the terms of Contract No. AT-(40-1)-1083 at Louisiana State University and Contract No. W-7405-eng-26 for the Atomic Energy Commission at Oak Ridge.

been described (3, 19). The fact that the development of single fungal spores, tagged with C^{14} and grown on nonradioactive medium for periods up to 48 hours, can be followed autoradiographically (19) is indicative of the sensitivity of this method. Results obtained with tritium indicate that, with this isotope, intracellular localization of radioactivity is possible through autoradiography (3). In the preparation of histological sections for autoradiographic assay, precautions must be taken to avoid movement or leaching of radioactivity during fixation and dehydration of the material. Autoradiography of histological sections of plants infected with tagged pathogens offers a means of demonstrating the presence or absence of radioactivity in cells showing disease symptoms but which have not been invaded by the pathogen. The absence of radioactivity in such cells would provide strong evidence that the symptoms were the result of host reaction products rather than of toxins produced by the pathogen. The presence of radioactivity, however, would not constitute conclusive proof that microbial toxins were involved because autoradiographs do not provide information concerning the chemical nature of the radioactive compounds present.

Assays of radioactivity in toxic fractions of extracts from plants inoculated with tagged pathogens should serve to distinguish metabolic products of the pathogen from reaction products of the host. Such separation would eliminate one of the most difficult problems involved in the isolation of microbial toxins produced *in vivo*. Specific methods for isolating toxins will, of course, depend upon the nature of the toxic agent and the training and experience of the investigator. One method which should prove generally useful is column or paper chromatography with radioactivity, rather than color, being employed to determine the location of labeled compounds. Paper chromatograms may be assayed autoradiographically by exposure to photographic emulsions (9, 12), areas showing radioactivity eluted, and the eluates tested for toxicity. Further separation may be achieved by chromatographing the toxic eluates.

SUMMARY

A direct test of the toxin theory of plant disease requires the development of methods by which toxins produced within host tissues can be detected and distinguished from host reaction products. The use of plant pathogens, tagged with appropriate radioisotopes, for the production of labeled toxins within the host has been suggested as an approach to this problem. Theoretical considerations and limited experimental data indicate that such an approach is feasible. Carbon^{14} and tritium (used as a tracer for carbon) appear to be the most generally useful radioisotopes for the production of labeled toxins. Autoradiography and chromatography appear promising as methods for the detection and assay of labeled toxins produced within host tissues by tagged plant pathogens.

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CONTROL OF TOBACCO DISEASES THROUGH RESISTANCE[1]

E. E. Clayton

Tobacco has been an important commercial crop in this country since 1612. At present it is a major source of cash income for some 500,000 farmers, and the United States produces almost 1/3 of the world's tobacco. During the early years of tobacco culture, the crop moved constantly to new areas and new land. Disease problems were not acute. About 50 years ago, however, production began to be stabilized and intensified in certain regions favored by soil and climate. This had great practical advantages, but it has been a major factor in the increase of disease problems. Critical situations have arisen because of black root rot (*Thielaviopsis basicola* (Berk. & Br.) Ferr.), wildfire (*Pseudomonas tabaci* (Wolf & Foster) F. L. Stevens), blue mold (*Peronospora tabacina* Adam), bacterial wilt (*Pseudomonas solanacearum* E. F. Sm.) and black shank (*Phytophthora parasitica* (Dast.) var. *nicotianae* (Breda de Haan) Tucker) as well as root knot (*Meloidogyne* sp.), mosaic and diseases of lesser importance.

The events of the past 40 years have clearly shown that the varieties of *Nicotiana tabacum* L. now grown as a crop, under conditions of specialized production, are far too susceptible to disease. Rotation, sanitation, and chemical control measures have been extensively tried. Alone or in combination they have proven inadequate. Resistance has been clearly demonstrated to be the best approach in coping with a series of very severe disease problems, and it may be described now as the foundation of the tobacco disease control program.

It is important to note that in combination with resistance other control measures tend to be more effective. The combination of rotation and resistance is more effective than either measure alone. Fungicides are needed for tobacco blue mold control and soil fumigants are finding a place in some areas. Resistance works best when supported intelligently by other disease control measures. It is very certain, however, that all tobacco growers everywhere want just as much disease control as possible through resistance.

The achievements of recent years through disease resistance have been outstanding. Much detail may be obtained from the excellent account recently published by Valleau (9). However, this presentation is not to be merely an account of successes. For one thing, research workers usually report positive results, and in the case of tobacco disease resistance the negative results, that have not been widely publicized, have great significance. Most people tend to assume that the outstanding progress of past years will continue unabated in the years ahead. This may be questioned. Perhaps a major criticism that can be made of many disease resistance breeding programs is that, after some outstanding results have been obtained, and after basic limitations of materials and methods have been revealed, there is still an extreme reluctance to change the program substantially. Some of the problems that have arisen in using resistance to control tobacco diseases will be discussed in this paper. Many pathologists have little immediate interest in tobacco, but tobacco is a typical seed propagated crop plant, and the findings will apply to many other crops.

In the following discussion resistance is used as an inclusive term, thus moderate or high resistance. Immunity is reserved for the situation in which a parasite is unable to reproduce and maintain itself, that is, freedom from disease. Neither term has any connection with parasite (or saprophyte) invasion, which can be temporarily facilitated by artificial conditions and has little significance. In practice it is not always easy to draw a line between high level resistance and immunity, and quite commonly varieties are referred to as resistant regardless of whether they are, strictly speaking, resistant or immune. Actually very little immunity has been found.

The modern tobacco disease resistance breeding program began with James Johnson about 1912. At this time the idea was generally accepted that tobacco was a soil exhausting crop and hence could only be grown in a long rotation. Johnson (6) proved that the trouble was black root rot and not soil exhaustion. He showed that some varieties were resistant to root rot and that resistant selections could be obtained in other varieties. He produced a number of good resistant varieties. Black root rot was soon found to be a very critical problem in Kentucky, and the burley varieties grown in that area 30 years ago were highly susceptible. Valleau (10) developed a series of root rot resistant types culminating in Kentucky 16, the leading burley variety today. Work on black root rot resistance continued to dominate the tobacco breeding programs until about 1930.

In 1924 there was an important new development. Black shank was identified in the Quincy, Florida area and a little later Tisdale (8) was able to solve this problem by crossing and selecting for resistance within local types. This original occurrence of black shank disease caused little national concern, since the disease was supposed to be limited to extreme southern regions, and since the area affected was only about 6,000 acres of cigar tobacco. However, black shank has spread and today it is a critical disease problem with both flue-cured and burley tobacco, types which are planted over an area of 1½ million acres. The black shank resistance developed in Florida has been used to produce flue-cured resistant types that have been of tremendous value to growers in the Carolinas and Virginia. This same resistance is now being incorporated into burley tobacco.

[1] An invitational paper presented at the Symposium on Disease Resistance at the 44th Annual Meeting of The American Phytopathological Society in Ithaca, N. Y., September 8, 1952.

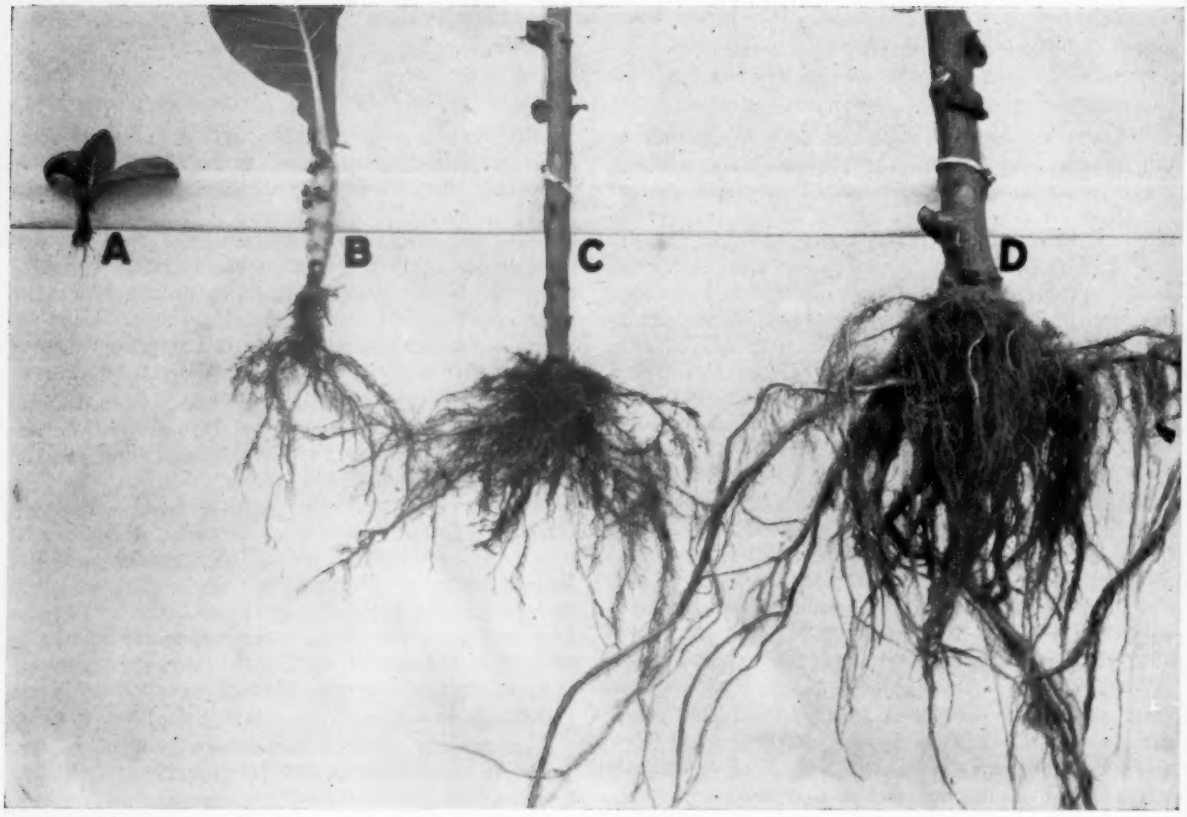

FIG. 1. Resistance and immunity to black root rot: A) susceptible variety; B) and C) resistant varieties; D) immune breeding line with root rot immunity from N. debneyi (Domin.).

A third outstanding achievement in developing disease resistant tobacco began in 1937 with the discovery of resistance to bacterial wilt in materials from Colombia, South America(3). This disease had defied the efforts of research workers for 30 years and was spreading steadily through the South. The release of wilt resistant varieties, beginning in 1944, has solved this problem.

Black root rot, black shank, and bacterial wilt resistance have in each case enabled growers to cope successfully with serious soil-borne diseases. The resistant varieties have made it possible for thousands of growers to continue profitable tobacco production. With susceptible varieties, these same growers often suffered losses of 25 per cent or more of their crop. Some of the important early resistant varieties released were root rot resistant Havana 142, black shank resistant Rg and Oxford 1, and wilt resistant Oxford 26. New and improved varieties are being added constantly.

The need for combinations of disease resistance has been recognized and recent introductions include Vamorr 50, resistant to root rot and mosaic; Dixie Bright 101, resistant to black shank, bacterial wilt and fusarium wilt (*Fusarium oxysporum* (Schlect.) var. *nicotianae* J. Johnson); and Kentucky 35 resistant to root rot, mosaic and fusarium wilt.

These developments convinced the tobacco growers of the great value of disease resistance.

However, more critical observers raise some questions:

1) Most of the disease resistant tobacco varieties are inferior to the better susceptible types, either in yield or quality or possibly both. Will this inferiority be overcome?

2) Many of the resistant varieties have only moderate resistance, and under very severe disease conditions can be seriously damaged. Will this damage increase?

3) In the case of root rot resistance, caused by a fungus with many physiologic races, some of the resistant releases are now quite subject to attack. Will there be a continuing breakdown of resistance?

All the resistance that has been discussed to this point has been obtained within the cultivated tobacco species. Logically the breeding work should have started with a comprehensive survey of all the *Nicotiana tabacum* types, but actually this was a practical impossibility. Until 1935 our knowledge of the total resistance available within this cultivated species was limited to the varieties grown commercially in this and other countries, plus a few additional collections. Tobacco, of course, was grown by the Indians before Columbus in parts of Mexico, Central America, and much of South America. It was in the 1934-35 period that this area of original distribution was systematically covered and some 1,000 seed collections obtained

FIG. 2. **Above**—commercial root rot resistant burley severely damaged by wildfire and mosaic inoculation. **Below**—a wildfire-mosaic resistant selection inoculated in the same way.

for study.

These collections were systematically indexed for resistance to major diseases using laboratory, greenhouse and field techniques. The evaluation work revealed 1) bacterial wilt resistance, as previously mentioned, 2) a number of collections highly resistant to black root rot, 3) a group of collections from Colombia and Venezuela with the Ambalema type of mosaic resistance, 4) root knot nematode resistance from Central America, and 5) a low level of blue mold resistance from Argentina. This makes an imposing list and all the resistance is within the cultivated species.

Since 1935 extensive work has been conducted with all of this resistant material. It seemed a natural assumption, at the time this work was started, that much of the resistance could be incorporated in part or in whole into commercial varieties. Table 1 shows the approximate "usability" of all the resistance found in *Nicotiana tabacum;* these estimates are based on 15 years or more of extensive work.

TABLE 1.—*Estimate of the disease resistance found in N. tabacum now incorporated in acceptable commercial varieties of tobacco*

Disease	Percentage of resistance incorporated
Root rot	50
Root knot	0
Black shank	60
Bacterial wilt	95
Fusarium wilt	95
Blue mold	0
Mosaic	0
Wildfire	0

Table 1 percentage figures represent estimates based on a large amount of testing. Taking root rot as an example, the resistance of the best collections is accepted as 100 per cent and, compared with this, the root rot resistant commercial varieties used in this country, average about 50 per cent resistant. Tobacco breeders have been forced to discard their more highly resistant selections because they were undesirable with respect to plant type, yield or quality.

Up to the present it has not been possible to use any root knot resistance in a commercial variety, and this despite extensive effort. The "percentage resistance incorporated" figure for root knot consequently is 0.

With the single exception of Ambalema mosaic resistance, all resistance listed in Table 1 is inherited in a polygenic manner. All of it has been recovered in the progeny of later generations after crossing with susceptible types, but the number of highly resistant segregates was usually small. The problem was that high resistance was consistently combined with poor yield, poor quality, or poor plant type. Many observers are inclined to assume that after a source of disease resistance is discovered, its utilization in a commercial variety is merely a matter of carrying out a certain routine of crossing and selection. It is commonly believed that by means of a systematic backcross procedure any resistance can ultimately be combined with practically any varietal type. Neither assumption has proven to be true for tobacco. Thus, the discovery of mosaic resistant Ambalema was announced in 1933 (7). Inheritance proved to be simple, digenic and recessive, but after numerous backcrosses to American types, all resistant selections were of undesirable plant type and no commercial variety with Ambalema resistance has been released.

Considering all the figures in Table 1 it is evident that on the average less than 40 per cent of available resistance has been incorporated into commercial varieties. The use of even this portion has involved some sacrifices of plant type, yield, or quality. With the 60 per cent not being used, the sacrifice was too great to be economically justifiable. Resistance at the expense of reduced returns per acre in absence of disease is a doubtful asset.

Will it be feasible to use more of the resistance available in *N. tabacum*, and will it be possible to improve present resistant varieties in other ways? These problems appear to be connected, in that resistance is usually inherited together with an inferior expression of other economically important plant characters. There are at least 2 adequate genetic explanations of this correlation. The problem may be "block" inheritance by which certain growth characters are consistently linked with certain genes for resistance. This mechanism, whereby character groups may be transmitted from generation to generation without recombination, has been aptly termed "total linkage." The other possibility is pleotropism, which would mean that certain resistance genes also affect the growth of the plant.

If either or both of these explanations is valid, the prospects are dim for further major progress with programs limited to the resistance that occurs within this cultivated species. However, work is continuing. Some investigators hope that with very large numbers of crosses and numerous segregating progenies, the linkage between poor plant type and resistance may be broken. Another proposal is to go back to original sources of resistance, and repeat some of the earlier work, in the hope of accumulating more of the desired genes. These efforts will continue but there is no indication that they will meet with any large degree of success. In fact for some years there has been a growing conviction that better resistance is the basic problem for the whole tobacco breeding program, and that this better resistance simply does not exist in *Nicotiana tabacum*.

Can it be obtained elsewhere? This question brings us to the other species in the genus *Nicotiana*, about 60 types of wild plants. Investigations during the 1930's showed that many of these are highly resistant or immune to major tobacco diseases.

The use of such resistance, however, has been consistently discouraged; and there is no precise information regarding procedures for interspecific transfer of disease resistance. The accounts of work with some vegetatively propagated plants suggest almost a random attack. Fortunately, we have now had enough experience with interspecific gene transfers in tobacco

to map a definite program. The researches with the *Nicotiana glutinosa* L. local lesion reaction to mosaic have been most helpful in this connection. This development began in 1925 with the report by Clausen and Goodspeed (1) of the first synthetic, fertile, allopolyploid species, a *glutinosa-tabacum* combination named *N. digluta.* A little later Holmes recognized the potentiality of this species as a source of mosaic resistance. He backcrossed *N. digluta* to tobacco and carried the local lesion reaction through a series of backcrosses. In 1938 he (5) made available to tobacco workers a line homozygous for the local lesion reaction.

However, when this material was used in an attempt to breed commercial mosaic resistant varieties, little immediate success was obtained. The mosaic resistant selections had consistently smaller leaves. It remained for Gerstel (4) to show that in the mosaic resistant tobacco an entire *glutinosa* chromosome had been substituted into the *tabacum* genome. His work indicated that it was possible to have a functioning tobacco plant with 24 bivalent chromosomes, 23 from tobacco and 1 from *glutinosa.* The leaf size of such plants was reduced, although the reduction was not conspicuous.

This problem was solved almost as soon as presented. Additional backcrosses to tobacco caused the intact *glutinosa* chromosome to disappear, resulting in normal appearing tobacco with the glutinosa mosaic reaction. Following this development there has been no difficulty in using this mosaic reaction and obtaining desirable commercial types. Some have already been released, as for example, Kentucky 35 and Vamorr 50. These varieties are truely mosaic immune since the virus is unable to maintain itself in the resistant plants and hence crops produced are completely free of mosaic virus. It seems probable that in a few years most tobacco varieties will be mosaic resistant.

The discoveries made over this period of 20 years, with the *glutinosa* mosaic resistance have given a true picture of the course that must be followed for other interspecific transfers. This series of steps in transferring the wildfire-blackfire immune reaction from *N. longiflora* Cav. to tobacco (2) is complete. Work is well advanced with transfers of black root rot immunity and blue mold resistance from *N. debneyi* Domin. These 4 interspecific gene transfers when completed will provide a higher level of resistance, which is more simply inherited, and hence much more easily incorporated into varieties, than anything that has been obtained within the cultivated species during the past 40 years; so far no difficult linkage problems have been encountered. More and more of the polygenic *N. tabacum* resistance will probably be discarded as better disease resistance transferred from related species becomes available. Present plans call for the use of resistance to 1) bacterial wilt, 2) fusarium wilt, and 3) brown spot (*Alternaria longipes* (Ell. & Ev.) Mason) from *N. tabacum;* 4) mosaic from *N. glutinosa,* 5) wildfire and 6) blackfire (*Pseudomonas angulata* (F & M) Holland) from *N. longiflora;* 7) black shank from *N. longiflora* and *N. plumbaginifolia* Viv.; 8) black root rot and 9) blue mold from *N. debneyi;* 10) root knot nematode from *N. megalosiphon* (Heurck and Muell.-Arg.). All 5 species mentioned have been successfully crossed with tobacco.

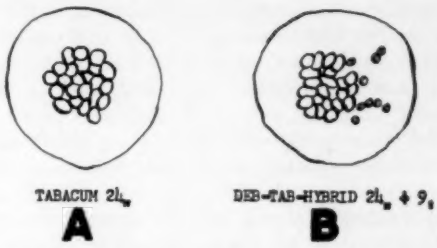

FIG. 3. A) Chromosomes from a tobacco plant showing the normal 24 bivalents. B) Chromosomes from a *tabacum-debneyi* hybrid plant that was blue mold resistant. The resistance was carried in the 9 univalent *debneyi* chromosomes and was expressed in the presence of a full set of 24 bivalent *tabacum* chromosomes.

In Table 2, in addition to the disease and the source of resistance, the level of the available resistance is indicated and its inheritance.

TABLE 2.—*Major Sources and Types of Resistance to Tobacco Diseases*

Disease		Resistance	
Leaf	*Source*	*Level*	*Inheritance*
Mosaic	*N. glutinosa*	Immunity	Monogenic
Wildfire	*N. longiflora*	Immunity	Monogenic
Blackfire	*N. longiflora*	Immunity	Monogenic
Brown Spot	*N. tabacum*	Mod. Res.	Polygenic
Blue Mold	*N. debneyi*	High Res.	Polygenic
Stalk			
Wilt-Bacterial	*N. tabacum*	Mod. Res.	Polygenic
Wilt-Fusarium	*N. tabacum*	Mod. Res.	Polygenic
Black Shank	*N. tabacum*	Mod. Res.	Polygenic
	N. longiflora	High Res.	?
	N. plumbaginifolia	High Res.	?
Root			
Root Rot	*N. tabacum*	Mod.-High Res.	Polygenic
	N. debneyi	Immunity	Monogenic
Root Knot	*N. tabacum*	Mod. Res.	Polygenic
	N. megalosiphon	High Res.	?

Some of the findings in the tobacco work are not in line with previous thinking on the subject of interspecific hybridization as a practical method of obtaining disease resistant cultivated plants. Vavilov (11), for example, concluded that with seed plants such as tobacco, crossing should be either confined to within the species, or at most extended only to closely related species. *Nicotiana* is a large genus, and some species are closely related to tobacco, but the valuable transfers of disease immune reactions have come from the species that are distant relatives, and so far nothing of value has been obtained from closely related species.

Another confusing problem is what to do after making an interspecific cross and a backcross to the cultivated plant. Some workers with other seed crops have spent years attempting to select and stabilize lines from first and second backcrosses, and even from the original cross. Their object has been to incorporate into the cultivated species entire chromosomes or even sets of chromosomes from the wild plant. This procedure has been successful with certain vegetatively propagated plants, but not with seed plants. During the course of our tobacco work it has been possible, after interspecific crossing, to obtain homozygous lines as early as the first backcross, but these lines have had no commercial value. They have been of unsatisfactory type and would present serious problems when crossed with regular tobacco varieties. Even one extra chromosome from the wild species has been too much for practical use, and it appears probable that even a large chromosome segment from the wild species may be too much. Repeated backcrosses to the cultivated plant brings about first, a translocation of the desired block of genes to a chromosome of the recurrent parent, and later a probable reduction in the size of the translocated segment to a point where it pairs normally as part of the tobacco genome, and regular Mendelian segregation results. It has only been after repeated backcrossing to tobacco, and after the 24 chromosome situation has been restored, that it has been possible to obtain commercial types that appear to be changed only with respect to 1 specific disease reaction.

Again, in crossing tobacco, which has 24 chromosomes, with other species, the original cross and subsequent transfer of resistance is no more difficult with *N. longiflora* (n=10) than with *N. debneyi* which has the same number of chromosomes as tobacco. In either case the normal 24 pair tobacco genome must be restored before anything of practical value is achieved.

Finally, it should be noted that interspecific transfers can be carried forward rapidly only with dominant types of resistance. Thus the *Nicotiana* species used have chromosomes that do not pair with the tobacco chromosomes, and during all early backcross stages the resistance is carried as extra monosomics, and the resistance genes must eventually act in the presence of a full 24 diploid tobacco set. Under such conditions recessive resistance would not be expressed.

SUMMARY

Resistance to the major tobacco diseases can be found in *N. tabacum*, but so far it has been possible to utilize less than half of this resistance in commercial tobacco varieties. The nonusable portion has been consistently associated with poor plant type, yield or quality. The inheritance of resistance obtained within *N. tabacum* is polygenic, with 1 exception. Despite these handicaps an entire series of valuable commercial varieties has been produced, carrying in varying degree resistance to black root rot, black shank, bacterial wilt and fusarium wilt.

Immunity or very high resistance to the major tobacco diseases has been found in various species of *Nicotiana*. Transfers of simple monogenic dominant immunity to mosaic and wildfire have been completed with no apparent association of this immunity with any other plant characters. Similar work is well advanced with root rot immunity and blue mold resistance.

All indications are that these transfers from distantly related species, once established in the tobacco genome, can be used far more rapidly and with fewer complications involving type, yield, and quality, than is the case with any resistance found within the cultivated species.

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CONTROL OF POTATO DISEASES BY DISEASE RESISTANCE [1]

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Resistance to a number of diseases in both the wild and cultivated species of potato has been found. More than 150 wild tuber-bearing species and at least 23 cultivated species of *Solanum* have been studied taxonomically and cytologically (12) but the work of evaluating them from the genetic standpoint has barely begun.

Cytologically potato species have been found in 5 groups with somatic chromosome numbers of 24, 36, 48, 60, and 72. The varieties belonging to *Solanum tuberosum L.* have 48 somatic chromosomes (40).

DISEASE AND INSECT RESISTANCE IN WILD SPECIES.—In recent years renewed interest in the wild Solanum species has resulted in a new interregional potato-introduction and preservation project at Sturgeon Bay, Wis. Its purpose is to procure and maintain species, species hybrids, and cultivated varieties of potato both domestic and foreign and to make the material available to geneticists, pathologists, entomologists, and others. A large worth-while collection has already been assembled, and a number of states and foreign countries are cooperating with the United States Department of Agriculture in evaluating the various introductions. At the present time the stocks are being tested for their reactions to Verticillium wilt (*Verticillium albo-atrum* Reinke & Berth.); *Fusarium* spp.; common scab (*Streptomyces scabies* Thaxt.) Waksman & Henrici); late blight (*Phytophthora infestans* (Mont.) DBy); early blight (*Alternaria solani* Ell. & G. Martin) Sor.); southern bacterial wilt (brown rot) (*Pseudomonas solanacearum* E. F. Sm.; ring rot (*Corynebacterium sepedonicum* (Spieck. & Kott.) Skapt. & Burk.); blackleg (*Erwinia carotovora* L. R. Jones) Holland); viruses A and X; and leaf roll. Cytological and crossability studies are also under way.

Previous studies have shown that a number of wild species possess characters of disease resistance of interest to geneticists and pathologists. Ross and Baerecke (29) reported immunity among these species from viruses X, B (closely related to X), Y, A, and leaf roll.

Immunity from X and B was found in a homozygous condition in some plants of *Solanum acaule* Bitt. Other plants of this species were heterozygous; when selfed they produced immune and non-immune seedlings.

Y-immunity was found in a number of species: *Solanum chacoense* Bitt.; *S. cordobense* Buk.; *S. garciae* Juz. & Buk.; *S. catarthrum* Juz.; *S. macolae* Buk.; *S. ajuscoense* Buk.; *S. antipovichi* Buk.; and *S. polyadenium* Greenm.; and possibly also in *S. demissum* Lindl.; *S. chaucha* Juz. & Buk.; *S. rybinii* Juz. & Buk.; and *S. commersonii* Dun. In crosses among these species and in a few hybrids with *S. tuberosum* immunity from virus Y was transmitted to the F_1 and F_2 generations.

Immunity from virus A seemed to be as general as immunity from Y. Immunity from leaf roll was not so common. The species *Solanum chacoense, S. catarthrum,* and *S. andigenum* Juz. & Buk. were the only ones showing immunity in some of their plants. All hybrids with *S. tuberosum,* except one with *S. chacoense,* showed leaf roll. *S. polyadenium* and its hybrids with *S. tuberosum* showed a high degree of tolerance for the leaf-roll virus. They seldom showed symptoms in the year of infection and possessed only very faint necrosis in the phloem.

In a report to the Scottish Society for Research in Plant Breeding (37) Cockerham reported immunity from virus Y in *Solanum rybinii* and (36) hypersentivity to virus Y in the diploid species, *S. simplicifolium* Bitt. in 2 clones of the pentaploid species, *S. salamanii* Hawkes and in 2 clones of the hexaploid species, *S. demissum.* Several progenies of parentage involving species of different chromosome numbers were raised. Many seedling varieties produced no tubers when grown in the field, but this and other "wild" characters have been eliminated by backcrossing to commercial varieties. Some seedlings of the backcrosses were found to be immune from virus Y.

For many years it has been known that some varieties of *Solanum demissum* were immune from late blight (30, 31). These have been used in breeding for immunity from late blight in Europe and America, and varieties related to this species that show resistance to the common races of Phytophthora have been released to growers. Previously unidentified new biologic races of the organism have been discovered in the United States, Canada, Scotland, Germany, and other European countries. The immune varieties of *S. demissum* are immune from all races so far studied, and recent seedling varieties, some of which have commercial promise, seem to be immune from all the races that have been identified in the United States. Rudorf et al. (30), working with 8 physiologic races, found the following species to be completely resistant to all biotypes: *S. polyadenium, S. verrucosum* Schlecht., *S. antipovichi, S. ajuscoense, S. demissum* f. *tlaxpehualcoense* Buk., *S. demissum* 029, *S. demissum* El Desierto Redd 530.

Resistance to common scab has been found in several wild species. According to Reddick (27), *S. commersonii, S. chacoense, S. caldasii* var. *glabrescens* Dun., and *S. jamesii* Torr. were grown in soil heavily infested with common scab organisms and for 2 successive years gave an immune reaction.

Resistance to leafhopper injury.—The leafhopper,

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Empoasca fabae (Harr.), is probably the most destructive insect attacking potatoes. Some of the wild species show a higher degree of resistance to hopperburn than any of the cultivated sorts. Sleesman (38) found that *Solanum chacoense*, *S. caldasii* Humb. & Bonpl., *S. commersonii*, and *S. polyadenium* were very lightly populated. *S. polyadenium* appeared to be almost immune from leafhopper attack. No first instar nymphs were observed, indicating that insects found on plants of this species probably migrated from adjacent susceptible varieties. In laboratory feeding tests all nymphs fed on *S. polyadenium* died, as did 88 per cent of those on *S. caldasii* and 63 per cent on *S. demissum*. In contrast, only 3 per cent of those on Irish Cobbler died.

Multiple resistance in the wild species.—Resistance to a number of diseases is found in some wild species. For example, *Solanum polyadenium*, according to Sleesman (39), is immune or nearly immune from leaf hopper injury, and, according to Rudorf et al. (30), it is completely resistant to all biotypes of *Phytophthora infestans* and is equally important as a resistant parent in selection for resistance to Colorado beetle and to virus.

The wild species could contribute much to the production of improved varieties but they have not been used extensively since most of them are deficient in horticultural characters, such as market and cooking qualities and high yield. Another deterrent to their more frequent use in breeding is the difficulty involved in getting hybrids. Some like *S. demissum* (2n=72), cross readily with *Solanum tuberosum* (2n=48), but many others appear incompatible. However, new technics may help to solve some of the problems. In a few cases incompatibility between a diploid and a tetraploid has been overcome by doubling the chromosome number of the diploid (2n=24) with colchicine (18, 44).

In spite of the handicaps, many species hybrids have been made but no commercial varieties, with the exception of those derived from the demissum-tuberosum crosses, have been released to growers.

DISEASE RESISTANCE IN CULTIVATED VARIETIES.—Although wild species will contribute much to genetic programs, the work of the future, as of the past, will always be more concerned with the cultivated potato varieties, because it is from these that we derive the most important horticultural characters. The cultivated varieties belong to the species commonly called *Solanum tuberosum* but many of them, according to Salaman (31), are related to *S. andigenum*. The 2 species seem to be closely related. Each has 48 somatic chromosomes and they are interfertile.

Since the introduction of potatoes into Europe, species hybrids have been made, so that *Solanum tuberosum*, as we know it, is heterogeneous—if indeed it can be called a species. The heterogeneity is shown by the many characters that have been found in it, among which are resistance to mild mosaic (virus A + virus X) latent mosaic (xirus X), rugose mosaic (virus X + virus Y), leaf roll, net necrosis, yellow dwarf, late blight of vines and tubers, common scab, wart, Verticillium wilt, ring rot, brown rot, and black leg.

Genetics of resistance.—The genetics of resistance to some of these diseases has been determined, and even though much has been learned about the breeding behavior of the reaction to other diseases, the number of genes involved is still unknown. The factors that often limit a genetic interpretation of disease resistance data in *Solanum tuberosum* include irregular segregations because of irregular chromosome behavior, absence of standardized disease tests, environmental effects, and biological races of causative organisms.

For many years it was supposed that the cultivated varieties of potato were functional diploids. The number of somatic chromosomes (2n=48) was determined by several investigators, and many irregularities in chromosome behavior were reported. Longley and Clark (19) studied cytologically 37 varieties of *Solanum tuberosum* grown in the United States. Chromosome behavior was frequently so variable that chromosome counts were made with difficulty. The somatic number of chromosomes was constant for all varieties but they differed from one another in the number of abnormalities observed in meiosis. In some varieties the 2 reduction divisions occurred but frequently unpaired chromosomes were found among the bivalents. In other varieties, some cells went through only 1 division and a number showed no chromosome pairing. The most irregular behavior was found in another group of varieties, the cells of which showed the somatic number of chromosomes, but true meiosis was frequently absent. The chromosome behavior of a few selected individuals seemed to be affected by environmental changes. In metaphase I most research workers have observed such irregularities as unpaired univalents and sometimes multivalent chromosome rings and chains. The multivalent groups were studied by Meurman and Rancken (22) who found groups of 3 to 6 chromosomes. From associations observed they concluded that the cultivated varieties of potato are fundamentally autotetraploid, but they observed that there were only 2 chromosomes with satellites. This finding indicates that some differentiation has taken place so that the potato now has a number of the characteristics of allotetraploids.

In recent studies of pollen mother cells Buck (8) found tetravalents, trivalents, bivalents, and univalents in the same cultivated variety, which, again, might indicate that some genes would segregate on the tetrasomic basis and others on the disomic basis.

The genetic evidence as to the nature of polyploidy in the potato is no more conclusive than the cytological. The earlier works regarded the plants as functional diploids, and the mode of inheritance was interpreted as disomic. Müller (24), in 1930, working with resistance to late blight, was perhaps the first to explain genetic results in the potato on a tetrasomic basis. Lunden's early writings interpreted the inheritance of genetic characters on a disomic basis but in 1937 as a result of a more extensive study based on large populations he (20) concluded that the segregations of the

genes with which he worked had to be explained on an autotetrasomic basis.

The choice lies between a disomic and a tetrasomic interpretation of inheritance; whereas some investigators adhere to the hypothesis that the potato is an allotetraploid, others have worked with segregating populations that can be explained best on the hypothesis that the potato is an autotetraploid. The difficulty of arriving at a definite conclusion may be due to the fact that *Solanum tuberosum* is an old polyploid, which may have undergone considerable change since it first came into being. It is probable that some of the groups of 4 chromosomes have become differentiated into 2 pairs so that their behavior is that found in an allotetraploid, whereas others have undergone little or no differentiation and continue to pair at random like 4 homologous chromosomes of a true autotetraploid.

Genetic interpretations have been impeded by a lack of standardization of disease tests. Immunity from a disease is not influenced by environment, but only a few immune characters have been found, such as immunity from wart, virus A, virus X, and late blight. On the other hand, there are many resistance characters that are influenced in their development by environment, and if the techniques of testing are changed from time to time the results are not comparable and can become very confusing. Physiologic races of a causative organism complicate the problem.

Breeding for resistance to a disease incited by a new race of a disease organism requires the same procedures as breeding for resistance to a new disease.

Assuming that the cultivated varieties of potatoes are autotetraploids, every character will behave in inheritance as a quantitative character behaves in an allotetraploid. If the character is controlled by a single gene pair Aa, 5 genotypes result: A_4, A_3a, A_2a_2, Aa_3, and a_4. If 2 pairs of genes are involved, 25 genotypes are produced. The number of phenotypes found will depend on the degree of dominance and dosage of the genes. Environmental factors also contribute to development of character so that the wide range of variability often found is difficult to interpret genetically. Nevertheless, some progress can be reported.

Resistance to virus diseases.—A list of the important virus diseases in the potato would include mild mosaic, latent mosaic, rugose mosaic, veinbanding mosaic, yellow dwarf, leaf roll, and net necrosis, the last caused by current-season infection with the leaf-roll virus. They occur in every potato-growing region in the United States; probably not a single field of potatoes could be found entirely free from one or more of them.

Mild Mosaic.—Mild mosaic is caused by the interaction of viruses A and X. A few varieties of potato, such as Irish Cobbler, were long known to be free from this disease because, as it was later discovered, they were immune from virus A. The differences observed between varieties with respect to their reaction to mild mosaic were made the basis of a systematic breeding program started about 1920 by the late C. F. Clark, horticulturist, U. S. Department of Agriculture. The work was started with the selection of seedlings that were free or comparatively free from mosaic when grown for a number of years in the potato-breeding plots on Aroostook Farm, Presque Isle, Maine, where at that time mild mosaic occurred on susceptible varieties in epidemic proportions.

Two seedlings, 24642 and 40568, were outstanding for their resistance. From a cross between these 2 the varieties Katahdin and Chippewa were selected, and after many tests they were released to growers (10). These 2 varieties have never contracted mild mosaic in the field since they were grown from seed in 1923.

Immunity from virus A in the field behaves as a dominant. Four hundred sixty-four seedlings of Katahdin selfed were tested for reaction to mild mosaic. Of these 93 per cent seemed to be immune and 7 per cent were susceptible. Some of the seedlings counted as immunes were possible escapes, but it is not likely that many were because 100 per cent of the Green Mountain checks contracted the disease (34). On a tetrasomic basis Katahdin is duplex for the gene controlling field immunity, although the selfed line produced too many susceptibles to give a close fit to the calculated ratio.

The early work of the Department of Agriculture with immunity was concerned with the reaction of varieties and seedlings to aphid infection with virus A in field-exposure tests. Recently it has been discovered that Irish Cobbler and some of its relatives, notably Earlaine, are immune from infection in the field and virtually immune in grafts; that is, if plants of these varieties are grafted onto others carrying virus A a hypersensitive reaction takes place on the field-immune plants, but the virus cannot be recovered in the tubers. The breeding behavior of the hypersensitive reaction is being studied but at present its mode of inheritance is unknown.

Latent mosaic.—Latent mosaic may be caused by any one of several strains of the virus X group, which are classified according to their virulence. The disease was called latent because it was found to be harbored in masked or latent form in such varieties as Green Mountain, Irish Cobbler, and Triumph. However, the symptoms are not always masked, as juice inoculations and grafts from masked carriers on certain other varieties result in top necrosis (34). Mosaic symptoms caused by virus X alone have been found on Mohawk, and Larson (17) reported a foliar mottle and necrosis in Chippewa potatoes as the result of infection by a strain of virus X. Mai (21) found virus X to be the cause of mosaic symptoms in potato plants of the varieties Katahdin, Sebago, and Chippewa, which were collected from fields in New York State.

In breeding for resistance to the various mosaic diseases, C. F. Clark crossed a selection of a selfed line of the South American variety Villaroela with U.S.D.A. Seedling 24642. One selection from this cross, designated S 41956, was resistant to mild mosaic in the field and, as was later shown by Schultz et al. (34), was immune from latent mosaic. Later tests and tests by other investigators have shown that this variety is immune in graft tests from all races of virus X

known at present. With S 41956 as a beginning, hundreds of seedling varieties immune from virus X have been bred. Some are promising from the commercial standpoint. Two of them, B 606-37 and B 606-67, are immune not only from virus X but also from virus A and are highly resistant to if not immune from the common races of the late blight fungus. In the Maine tests they produce high yields of tubers equally high in dry-matter content as Green Mountain. They are being increased, and if they continue to show promise one or both may be released to growers.

The inheritance of immunity from virus X in graft tests was studied (43) in 3 progenies of immunes selfed and crosses between immune S 41956 and 2 non-immune varieties. Of the selfed lines, 72–78 per cent and of the progenies of the 2 crosses 37 per cent were found to be immune. The results were explained by assuming autotetrasomic inheritance in the potato with the genes A and B both necessary to give immunity, the immune plants having the genetic constitution $A_2a_2Bb_3$ and the non-immune, a_4b_4. Recent observations indicate that the hypothesis may need modification, but the information obtained has been very helpful to the potato breeder because it is apparent that a relatively high percentage of the progeny produced by selfing an immune will be immune and that immunes are to be found in the first generations of crosses between immunes and non-immunes.

Veinbanding mosaic (Virus Y).—Virus Y is the cause of veinbanding mosaic (35), one of the 3 major potato mosaic diseases. In susceptible varieties virus Y has been known to cause at least a 50 per cent reduction in yield, whereas viruses A and X are each responsible for 15 to 30 per cent losses. Virus Y, in combination with virus X, causes rugose mosaic, a more severe disease on many varieties than that produced by any single virus.

Some cultivated varieties, such as Katahdin and Chippewa, show a degree of resistance to virus Y in field-exposure tests. Selfed lines of these varieties indicate that this resistance is heritable but the number of genes involved has not been determined. Some of the seedlings were more resistant than the parents (35). They contracted virus Y in 5–10 per cent of the hills, whereas almost 100 per cent of the Green Mountain checks became infected. Resistance to infection by virus Y is related to aphid dosage. Varieties seemingly resistant in field-exposure tests are not necessarily resistant under heavy aphid infestation in cloth cages.

Hutton and Bald (13) reported that breeding for a hypersensitive reaction to virus Y seems to be more promising than breeding for immunity. The varieties Snowflake, Katahdin, and Brown's River were given as promising sources of hypersensitivity to virus Y. The hypersensitive reaction appears to depend on one or more recessive alleles, tolerance to the virus being dominant.

Leaf roll.—Many domestic and foreign varieties of potatoes and thousands of seedling varieties have been tested for resistance to leaf roll. Tests have been conducted at the Plant Industry Station, Beltsville, Md., at the Aroostook Farm, Presque Isle, Maine, and at the Highmoor Farm, Monmouth, Maine. Tests have been carried on also at the Washington State Irrigation Experiment Station, Prosser, Wash., and at other state experiment stations. The early work at Beltsville, Md., and at Presque Isle, Maine, consisted of tuber-graft and field-exposure tests. As none of the varieties or seedlings showed resistance in the tuber-graft tests they were discontinued. The early field-exposure tests on Aroostook Farm were unsatisfactory, because in some seasons there was very little spread of leaf roll. It was found that there was a consistent spread of the disease at Highmoor Farm; so about 12 years ago much of the testing work was transferred to that location in cooperation with Donald Folsom of the Maine Agricultural Experiment Station. A few years later the field-exposure tests were undertaken again on Aroostook Farm. This time the spread of leaf roll was assured by growing the aphid vectors in the greenhouse, feeding them on potato plants harboring leaf roll virus, and placing them on the plants in the test plots in the field. These tests are made by G. W. Simpson and Reiner Bonde of the Maine Agricultural Experiment Station.

A number of foreign and domestic named varieties and thousands of seedling varieties have been exposed to infection (41) but all have failed to show immunity from leaf roll. Foreign varieties, such as Friso, West Brabander, Triumf, Bevelander, Imperia, and Kepplestone Kidney, which were introduced because of their reported resistance to leaf roll, do not seem to become infected so quickly as Green Mountain, but under continued exposure they sooner or later contract the disease. The Dutch variety Triumf seems to be the most resistant. Among the American varieties Houma shows a high degree of resistance and Katahdin an intermediate reaction. Among the most promising progenies tested at Highmoor Farm were Kepplestone Kidney × Earlaine, Imperia × Earlaine, and Houma × Katahdin. In late years crosses between selections made at Highmoor Farm have shown a higher degree of resistance than the original crosses. Some seedlings have escaped infection for a number of years in the tests on Aroostook Farm, where viruliferous aphids were put on the plants. Those surviving several tests usually come from crosses related to Imperia or Kepplestone Kidney, but occasionally another source of resistance is found.

B 24-58, a selection from the cross Imperia × Earlaine, is resistant to leaf roll. In 8 years of exposure to the disease on Highmoor Farm it averaged 7 per cent infection. In 1951 it showed 13 per cent leaf roll, whereas Green Mountain and Chippewa showed 99 and 98 per cent, respectively. X 1276-185, a selection from the cross of Houma × Katahdin, has shown resistance in a number of tests. Another seedling variety, X 927-3, selected from the cross AAP-9 × Katahdin, has shown no leaf roll since it was first grown in 1940. AAP-9 is one of Donald Reddick of Cornell University's older seedlings that had 2 wild species,

Solanum maglia and *S. demissum,* in its ancestry. It is impossible to say from which ancestor X 927-3 inherited its resistance but up to the present time it has shown a near immune reaction in field, aphid and graft tests. The genetics of resistance has not been determined.

Selfed lines and crosses are now being studied on Aroostook Farm in the field-exposure tests with viruliferous aphids added in an attempt to determine the number of genes involved. In conjunction with these genetic studies an effort is being made to produce commercial varieties with a higher degree of resistance to leaf roll than that of any of the current commercial sorts.

Net necrosis.—Net necrosis, the result of current-season infection with the leaf roll virus, has caused severe losses in some of our highest quality varieties, such as Green Mountain and Russet Burbank. It produces a network of brown to brownish-black strands, usually confined to a small portion of the flesh near the stem end of the tuber, but if the potato is severely affected the strands may extend from the stem to the bud end, often following the fibro-vascular bundles. Net necrosis seldom appears in tubers until they have been in storage several weeks, and it reaches its maximum development several months after the potatoes have been dug. Net necrosis is not transmitted from the seed piece to tubers of the new crop, but spindling sprouts usually originate from that part of the tuber showing the disease, and plants from affected tubers may develop leaf roll. Since leaf roll is transmitted in tubers, the control of net necrosis consists of the use of certified seed free of the malady. Seed should be inspected carefully at cutting time, and if it contains more than a trace of tubers with net necrosis it should be replaced by seed containing none. The use of immune varieties is the best measure of control. Although no commercial varieties have been found that are immune from leaf roll, a number are highly resistant to if not immune from net necrosis. In this group are Chippewa, Katahdin, Sebago, Houma, and Kennebec. These varieties have solved the net necrosis problems of the growers in Maine, where the old standard Green Mountain variety can no longer be grown profitably in some sections because of this disease.

The genetics of resistance to net necrosis is unknown, but it occurs far more often than leaf-roll resistance in the foliage.

Yellow dwarf.—As reported by Barrus and Chupp (3) yellow dwarf was first discovered by Barrus in the summer of 1917 in a field of Green Mountain potatoes in Clinton, New York. In 1922 it had been located in 20 counties and in 18 different varieties of potatoes, none of which showed any degree of resistance.

Since that time the disease has been reported in a number of other States including Florida, Michigan, Minnesota, New Jersey, Ohio, Pennsylvania, Vermont, and Wisconsin. Muncie (25) reported that losses in individual fields in Michigan often were 15 to 25 per cent. In a few cases the yield was decreased 75 to 90 per cent. Walker and Larson (46) stated that in 1935 in Washington County, Wisconsin, many fields were almost a total loss because of yellow dwarf, and in 1937 this disease was more destructive in the State than in any other year on record. Some varieties are resistant to attacks of yellow dwarf. Russet Burbank tended to escape infection in the section of central Wisconsin where 18 other varieties or strains of potatoes became heavily infected in the epidemic of 1937.

In tests made by Rieman and McFarlane (28) the Sebago and Russet Rural varieties were compared for yellow dwarf resistance on 19 representative farms in the yellow-dwarf area of central Wisconsin. Sebago showed a high degree of resistance to this disease in the field. Only ½ of 1 per cent of the plants of Sebago grown on 19 farms became infected, whereas approximately 18 per cent of the Russet Rural plants contracted the disease. The first-generation progeny of a cross between the resistant Sebago and the susceptible Hindenburg showed 7 per cent infection, whereas the Russet Rural checks were 40 per cent diseased.

Wheeler (48) reported that a potato yellow-dwarf epidemic was prevalent in the potato fields of Michigan in 1931 and 1932. All the commercial varieties grown showed a high percentage of the disease and 50 per cent of the seedlings in the plots at Lake City, Mich., were eliminated during the summer and fall of 1932. Thirty-seven promising seedlings that had escaped infection in the field were tested the following year with plug-graft inoculations. About 50 per cent of these escaped infection, indicating resistance in field-exposure tests and in graft tests.

Breeding with the definite purpose of producing varieties resistant to yellow dwarf has so far not been carried out to any great extent in this country, and no results of economic value have been produced. The Sebago was distributed for its blight resistance and other characters of importance, and its resistance to yellow dwarf was discovered after it had gone into commercial production. However, results of tests show that the disease can be controlled, at least in part, by the use of resistant varieties.

Late blight.—Late blight is so common in the humid sections of the United States best suited for potato culture and causes such heavy losses that it has been referred to as "the potato disease." In recent years fungicidal programs have been improved, but as recently as 1951 heavy epidemics of late blight, prevalent in many potato-producing areas, resulted in a potato shortage in the spring and summer of 1952. If carefully carried out spray programs are usually effective in controlling late blight but when heavy rains, such as occurred in Maine and other states in 1951, interfere with preventive measures heavy losses in yield and in stored tubers of susceptible varieties prevail. Under such conditions blight-resistant varieties are especially valuable.

In the United States and in foreign countries varieties of potato immune from late blight have been sought for more than 100 years. The problem of breeding such varieties has many complications.

There are a number of distinct physiologic races of

Phytophthora infestans. There seems to be no immunity in the cultivated varieties, and most of them are highly susceptible to all races. Immunity from all known races of late blight is found in wild species possessing no horticultural characters of value. Most of the immune wild species can not be crossed with cultivated varieties. Immunity from all races is dependent on multiple genes. Environmental factors cause variability in varietal reactions (42).

In spite of these complications, progress is evident in our greater knowledge of the inheritance of resistance and immunity and in the information we have on physiologic specialization in the organism. An intermediate degree of resistance was found in a selfed line of a susceptible cultivated variety and in a cross between 2 susceptible varieties. A genetic explanation might be that these varieties were carrying a gene for resistance in th simplex condition Aa_3 and that in the breeding process a number of duplexes were produced that showed some resistance to late blight. A somewhat higher degree of resistance was obtained when varieties intermediate for resistance were used as parents, but no immunity has yet been found in *Solanum tuberosum.* Several wild species of *Solanum* are immune from late blight but most is known about the immunity of *S. demissum.* This immunity is not inherited as a unit, as was once supposed, but is controlled by multiple factors. This fact has been determined not from genetic ratios found in segregating populations but by the reactions of seedlings that are related to *S. demissum* to different races of the late blight organism. Mills (23) stated that 3 distinct genes for resistance have been identified in the descendants of *S. demissum.* Intercrossing of plants carrying different genes will produce immunity to all races of the fungus, which have been found in the field. Black (4) found that immunity in *S. demissum* is controlled by 4 main genetic factors and several minor genes. Multiple genes for immunity found in *S. demissum* segregate in crosses and backcrosses with cultivated potatoes. Some of the resulting seedling varieties carry only 1 gene; others possess 2 or 3. These seedling varieties are far superior to the wild parent in horticultural characters. By using these improved strains as parents we are attempting to recombine the several immunity genes and to produce commercial varieties that are immune from all the physiologic races of late blight fungus in the United States. Immune varieties that meet most of the requirements are now under test at several state experiment stations, such as Cornell, Pennsylvania, and Maine.

Resistance of tubers to dry rot initiated by the late blight fungus is often more important than the foliage reaction. This is especially true in varieties having an intermediate type of foliage resistance. Varieties moderately resistant have been known to contract a light infection on their foliage; not enough to warrant spraying but enough to cause infection of the tubers and to result in heavy losses from late blight rot.

Bonde et al. (6) emphasized resistance to late blight in the tubers of cultivated varieties and seedling progenies. A high percentage of the seedlings were resistant to tuber and foliage infection when 1 or more parents were resistant. Some seedlings with resistant foliage produced susceptible tubers, and vice versa, indicating that foliage resistance and tuber resistance are controlled by different genes.

Uninjured tubers may be resistant but tubers of the same variety damaged by mechanical or other means may be susceptible to late blight rot. The mode of inheritance of resistance to late blight tuber rot has not been determined.

Common scab.—Many domestic and foreign varieties have been tested for reaction to common scab. Most old American varieties are very susceptible but a few, such as Russet Burbank, Russet Rural, and White Rose, showed a moderate degree of resistance. Foreign varieties, such as Hindenburg, Jubel, Ostragis, Rheingold, Arnica, and Ackersegen, vary somewhat in reactions but all are more highly resistant than the old American varieties. None is immune or has any commercial promise in the United States but when used as parents in crosses with domestic sorts many scab-resistant varieties have been produced. Five of these, Menominee, Ontario, Seneca, Cayuga, and Cherokee, have been released to growers.

Genetic interpretations of resistance to common scab in the potato do not account for all the variability encountered, part of which is due not only to the interaction and dosage of the alleles involved but to environmental factors and physiologic races of the causative organism. However, a number of factors that are helpful to potato breeders have been demonstrated. Clark et al. (9) reported that in tests made on the Aroostook Farm, Presque Isle, Maine, Green Mountain bred true for susceptibility. Katahdin was susceptible but carried at least 1 gene in a heterozygous state or, according to present interpretations, Katahdin is simplex, susceptibility being dominant in this condition. Hindenburg and Ostragis were probably homozygous for resistance. A russet-skin seedling, No. 44537, and Jubel were heterozygous. There was an incomplete linkage between russeting and resistance. In 1 cross the resistance was independent of red and white tuber colors. Russet, red, and white skin types highly resistant to scab were produced.

Krantz and Eide (15) assumed that inheritance in the potato is autotetrasomic and that the differences observed in reaction to common scab were controlled by 1 set of alleles Sc sc. Five phenotypes were found corresponding to the 5 genotypes that would be expected—Sc_4, Sc_3, sc Sc_2 sc_2, Sc sc_3, and sc_4. The phenotypes were represented by the varieties Hindenburg, Jubel, several unnamed varieties, Earlaine, and Chippewa.

As indicated above, some wild species of *Solanum* seem to be immune from scab, and these no doubt will be used in future breeding work. In the meantime, resistant varieties, such as Ontario and Cherokee, have been and will be of great value to growers whose land has become so heavily infested with scab organisms that the old susceptible varieties can no longer be

grown with profit.

Wart.—Wart incited by *Synchytrium endobioticum* (Schilb.) Perc. is an important disease in most countries of western Europe. It is not important in the United States, although it was discovered in mining villages in Pennsylvania in 1918 (16) and later in similar districts in Maryland and Virginia. It was reported in the northern midlands of England as early as 1876 (31) but not until 30 years later did its inroads cause any concern. Fortunately, a relatively large number of European varieties were found to be immune, and they probably saved the potato industry of a number of European countries.

In the United States wart is still confined to the restricted areas where it was first found. Its spread has been controlled by strict regulations governing the importation of foreign potatoes, by the growing of immune varieties, and by the laws prohobiting the shipment of potatoes out of the infested areas.

Orton and Weiss (26) tested a number of seedling varieties for reaction to wart and found a range from complete immunity to complete susceptibility. In another test by Weiss et al. (47) Irish Cobbler and Green Mountain were classified as immune but all varieties of the Rural group were highly susceptible.

Hartman and Akeley (11) reported on a series of tests that have been conducted over a period of years in Pennsylvania on new varieties and breeding cultures. Katahdin, Mohawk, Sequoia, Mesaba, and Norkota showed an immune reaction, and Houma was highly resistant. Of 60 other seedling varieties tested, valuable as parents and potential commercial varieties, 11 were immune, 4 very resistant, and 45 susceptible. About 50 per cent of a selfed line of Katahdin was very resistant to wart, indicating that resistance is dominant.

The first genetic studies of immunity from wart were made in England by Salaman and Lesley (32, 33). They found 4 genotypes among the immunes. One was homozygous and the other 3 segregated for immunes and susceptibles in the ratios of 3:1, 15:1, and 9:7. These ratios would indicate disomic inheritance with 3 factors involved, duplicate factors X and Y and a complementary factor Z. A study of the susceptibles showed that 1 or possibly 2 inhibitors were sometimes present. Other Europeans have made similar studies. Lunden (20) studied the inheritance of reaction to potato wart in 7,000 plants. Assuming tetrasomic inheritance he presented the following hypothesis: X, a dominant factor that brings out immunity even in the simplex condition; Y and Z, 2 complementary factors that give immunity when both are present even in the simplex state.

Verticillium wilt.—Verticillium wilt, which is incited by a soil- and seed-borne organism, is an old disease of potatoes. It has been called the "die early" disease in the Pacific Northwest. For a number of years it has been causing much damage in certain districts of California, Idaho, and Washington, and recently it has been reported in Maine and other potato-growing sections.

In the upper Snake River Valley of eastern Idaho, early planted fields of potatoes are often 70 to 80 per cent diseased by the middle of August, and the plants are dead in early September. The losses in certain sections amount to 30 to 50 per cent of the crop.

In 1949 a potato-breeding project, one of the main objectives of which is breeding for resistance to Verticillium wilt, was begun at Aberdeen, Idaho. Domestic and foreign varieties, parents, and progeny lines that were sent from Beltsville, Md., and Presque Isle, Maine, were tested on wilt-sick soil. Several named varieties and a number of seedling varieties have shown resistance. Among the most resistant or tolerant varieties are Sequoia, Menominee, Saranac, and Populaire. Green Mountain, Mohawk, and Sebago showed an intermediate type of resistance, and Irish Cobbler, Russet Burbank, Kennebec, and White Rose were very susceptible.

Resistance is reflected in the yields. In 1951 Sequoia, grown on wilt-sick soil and with an average Verticillium wilt index of 1.6, yielded 405 bu. per acre; Menominee with an index of 18.3 yielded 309 bu. per acre; and Russet Burbank with an index of 61.9 yielded about 209 bu. per acre. The differences are of course due in part to inherent yielding ability of the varieties.

Resistance is heritable but the number of factors involved is undetermined. The seedlings of a cross of 2 susceptible varieties showed a wide range of reactions from resistance to a high degree of susceptibility. In contrast, all seedlings of a cross between 2 resistant varieties were resistant, more than 70 per cent being in the most resistant class.

Ring rot.—Ring rot has been considered a menace to potato growing wherever it has been found. It was discovered in Germany early in the present century, and described by Spieckermann in 1913. In 1931 it was reported by Baribeau (2) as prevalent in potato fields in the Province of Quebec and a year later it was discovered in a field of potatoes in Aroostook County, Maine (5). By 1940 it had been found in 37 states and in some instances the losses from its inroads were very great (45). It has been held in check by enforcing a zero tolerance for certified seed and taking all other precautionary sanitary measures.

As soon as ring rot was recognized as a disease of major importance in the United States a survey was made of available varieties and seedlings and a high degree of resistance was found in the varieties Friso and President from the Netherlands and in a number of unrelated seedling varieties in the United States (7). Two of the latter, Teton and Saranac, subsequently were released to growers.

Under experimental conditions where the freshly cut seed pieces were smeared with bacterial ooze from infected tubers and then submerged in a water suspension of bacteria from the same source no ring rot was found in Teton in 3 out of 6 years. During the other 3 years it showed 1, 3, and 18 per cent infection. In contrast, comparable Green Mountain and Katahdin checks showed 65 to 100 per cent infection.

Many crosses and selfed lines have been tested. Selfed lines of the resistant varieties President, Saranac (President × Katahdin), and Teton produced, respectively, 55, 79, and 85 per cent resistant seedlings. None of the latter contracted ring rot in a test in which none of the 500 Katahdin controls escaped.

It is evident that resistance is dominant but the number of genes involved is undetermined.

Southern bacterial wilt.—Southern bacterial wilt, known also as bacterial wilt and brown rot, occurs in warm regions throughout the world. It is present in certain localities in the South Atlantic and Gulf Coast States from New Jersey to Texas. The bacteria are seed- and soil-borne but most of the disease is incited by the soil-borne organisms, as certified seed potatoes are usually produced in localities where brown rot does not occur. Reductions in yield of marketable potatoes often result.

No high degree of resistance has been found in the cultivated varieties. Katahdin and Sebago are less susceptible than other commonly grown varieties but their degree of resistance is of little or no value on heavily infested soils. A breeding program conducted in North Carolina has brought out differences in reaction to brown rot among varieties of *Solanum tuberosum* but so far none that will solve the problem in sections where conditions are most favorable for the development of the disease has been tested. At present, some wild species are being evaluated for their reaction to brown rot but no high degree of resistance can be reported at this time.

Blackleg.—Blackleg is the most common bacterial disease of potatoes. It is most destructive when the soil remains wet during the planting period. Under normal conditions the disease occurs here and there throughout the field, seldom affecting more than 15 per cent of the plants and usually less than 5 per cent. It is transmitted through infected seed potatoes but most of the infection is caused by soil-borne bacteria.

Properly suberized seed pieces are the best protection against blackleg. Varieties vary in reaction to this disease. Some of the American varieties, such as Sebago, seem to be more susceptible than others but controlled tests of available varieties have not been made. A few varieties that are reported to have varying degrees of resistance, such as Flava, Prisca, Robusta, and Starkeragis, have been introduced from Europe. Flava and Starkeragis have been selfed and crossed with other varieties but little resistance was found in any of the progenies. However, the test might be considered rather severe, as a bacterial suspension was injected into the eyes of the seed pieces with a hypodermic needle. They were planted in the greenhouse and were grown under conditions favoring blackleg. Different results might have been obtained under field conditions.

Hopperburn.—Hyslop (14) reported that in Wisconsin, Ohio, Illinois, and Michigan annual losses due to hopperburn were approximately 12,000,000 bu. The situation has greatly improved since the use of DDT has become more general but losses continue to occur.

Some varieties of *Solanum tuberosum* are more resistant to hopperburn than others but none that has been tested is immune. Allen and Rieman (1) found that in general the early maturing varieties were more susceptible than the late. Triumph, Warba, Earlaine, and Irish Cobbler were highly susceptible. Rural, Houma, Katahdin, and Russet Rural showed some degree of resistance.

In breeding for resistance to hopperburn Sleesman and Stevenson (39) found a number of seedling varieties that were much more resistant than any of the old commercial varieties. Sequoia, Sebago, Katahdin, and Rural and 61 numbered seedlings were significantly more resistant than Irish Cobbler, Warba, Triumph, Earlaine, and Pontiac. Sequoia was distributed to growers because of its resistance to hopperburn; although much work has been done on developing resistant varieties in recent years, Sequoia is still the outstanding commercial variety in its resistance to leafhopper injury.

Many attempts have been made to combine the immunity found in *Solanum polyadenium* with the horticultural characters of commercial varieties but little progress can be reported. Recently seedlings have been grown from seed[3] of a number of hybrids related to *Solanum polyadenium* and are now being tested for their reaction to hopperburn and late blight. Even if immunity from these 2 diseases is found in the seedlings it will take several generations of backcrossing to cultivated potatoes to produce a variety that will compete with present commercial varieties. However, that is one of the objectives of the present potato-breeding program. The inheritance of resistance has not been determined.

Multiple Resistance in Cultivated Varieties.—One of the main objectives of the present potato-breeding program is to combine in 1 variety resistance to as many diseases as possible. Seedling varieties have been produced possessing resistance to 2, 3, 4, and 5 major diseases. The last, B595-76, shows an immune reaction to viruses A and X, it is immune or nearly immune from the common forms of late blight, highly resistant to common scab, and tolerant to *Verticillium* wilt. The combination of the first 4 was produced by planned crosses and tests; the tolerance to *Verticilium* wilt occurred by chance.

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[3] Received through the courtesy of Wilhelm Rudorf, Max-Planck Institute, Voldagsen, Germany.

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BREEDING FOR DISEASE RESISTANCE IN CELERY AND CUCURBITS [1]

H. M. Munger and A. G. Newhall

In discussing breeding for disease resistance in celery and the cucurbits our main objects will be to answer 2 questions about each disease—what are the best sources of resistance to use in a breeding program and how readily can resistance be transferred to another variety? We shall not attempt to review those reports of varietal differences in resistance which do not give information relating to the above questions.

CELERY.—The chief diseases of celery which have, to some degree, been controlled by breeding are yellows (caused by *Fusarium oxysporum* f. *apii* (R. Nelson & Sherb.) Snyder and Hansen), early blight (caused by *Cercospora apii* Fres.), and late blight (caused by *Septoria apii-graveolentis* Dorogen).

Yellows.—Fusarium yellows became important in Michigan and New York between 1912 and 1920, during which time Nelson selected a resistant strain of Dwarf Golden Self Blanching from a commercial variety of that name (27). By the same method of individual plant selection in Golden Plume he later produced a resistant strain called Michigan Golden (28). In 1951 Nelson (26) released Michigan State Green-Gold, a yellows resistant variety selected from a cross between Michigan Golden and the Downing strain of yellows susceptible Fordhook. Strains of Golden Plume resistant to yellows have also been developed by seedsmen through selection and selfing.

In general, the green varieties of celery are resistant to yellows but an occasional green variety is susceptible. Resistance has been transferred from green to yellow varieties by crossing as in Cornell 6 and Cornell 19 for which Utah was used as the resistant parent. There is little published information on the inheritance of yellows resistance. It appears to be relatively simple although there is probably more than 1 gene involved.

Blights.—Breeding for resistance to early blight in celery was made possible by O. H. Pearson's introduction in 1936 of a Danish celery with very high resistance. He crossed this with Golden Self Blanching and gave the seed to Cornell breeders who were already working on yellows resistance. Successive crosses of Pearson's hybrid with Cornell 19 and Cornell 6 resulted in the release in 1951 of Emerson Pascal, a green celery resistant to yellows, moderately resistant to early blight, and unintentionally, somewhat resistant to late blight.

Townsend, Emerson, and Newhall (35) also found early blight resistance in several celeriacs introduced from Turkey, the best of which were PEI 115,557 and PEI 120,875. Subsequently these caleriacs and the Danish celery used previously were found to be resistant to late blight as well. The Danish celery seems slightly more resistant to early blight and the Turkish celeriacs slightly more resistant to late blight. However, there is very little difference in their value as sources of resistance to either disease.

In using either of these parents it has been difficult to maintain resistance at a satisfactory level because so many plants selected with small numbers of blight lesions turn out to have been lightly infected or escapes. With 2 completely different sources of resistance, the possibility of combining genes from each to develop more resistant or even immune celery presented itself. Following a cross between the two, distinct segregation has been observed and some F_3 progenies seem more resistant to early blight than either parent.

WATERMELON.—*Fusarium wilt.*—Breeding cucurbits for disease resistance had its beginning soon after 1900 with the work of Orton (29) on fusarium wilt resistance in watermelons. In this classical work Orton found resistance to *Fusarium oxysporum* f. *niveum* (E.F. Sm.) Snyder and Hansen, in the inedible citron. By crossing a citron with a wilt-susceptible but edible watermelon, he combined wilt resistance and edibility in the variety Conqueror. Since that time, more has been published on breeding for wilt resistance in watermelons than for any other disease of cucurbits. This subject has been reviewed in detail by Parris (30) and only a few points need be mentioned here.

Wilt resistance has been found not only in citron but in occasional plants of several commercial watermelon varieties. These include Kleckley Sweet, Grey Monarch, and Klondike. Pride of Muscatine, Leesburg, and Hawkesbury are wilt resistant varieties which have been produced by selecting such resistant plants. Later a number of varieties with improved horticultural characters have been developed by hybridization and selection. Some derive their resistance directly or indirectly from Conqueror, but more of them seem to get it from Leesburg and Hawkesbury.

Immunity to wilt has not been reported and even the most resistant varieties show about 20 per cent of diseased plants. There is evidence that all watermelons studied can become infected but that the fungus does not develop sufficiently in most of the resistant plants to bring about symptoms of wilt. The inheritance of resistance has been difficult to study, but apparently a number of genes are involved and resistance tends to be recessive. At one time, physiologic races of *F. oxysporum* f. *niveum* were suspected. However, most varieties developed in one state have been resistant wherever tested and it appears from this that physiologic races have not been a serious factor in breeding wilt resistant watermelons.

Anthracnose.—Anthracnose cause by *Colletotrichum lagenarium* (Pass.) Ell. and Halst. is the only other watermelon disease on which there has been much

[1] An invitational paper presented at the Symposium on Disease Resistance at the 44th Annual Meeting of The American Phytopathological Society in Ithaca, N. Y., September 8, 1952.

work in breeding for resistance. Layton (22) discovered the only reported source of resistance in African watermelons obtained from a missionary. These melons were not immune but resistance was much higher than any found in American varieties, infection being limited to small lesions. Layton's data indicated that the African watermelons differed from susceptible varieties by a single dominant gene for resistance. The anthracnose resistant African watermelons were crossed with wilt resistant varieties developed in Iowa, and an inbred line selected in Florida from this was crossed with Garrison at the U. S. Vegetable Breeding Laboratory at Charleston, S. C. An anthracnose resistant selection from this second cross was named Congo (1), and its rapidly increasing popularity attests to the value of anthracnose resistance as well as to its good horticultural characteristics.

MUSKMELONS.—*Powdery Mildew.*—The well-known work on powdery mildew resistance in muskmelons was started in 1926 (19). Resistance was found in a variety from India and appeared to be inherited as a single dominant factor (20). Powdery Mildew Resistant Cantaloupe No. 45, released in 1935, was selected from a cross between Hales Best and the resistant Indian variety. In 1938, there appeared a new race of the causal fungus, *Erysiphe cichoracearum* DC, and all commercial varieties including PMR 45 were susceptible (32). Resistance to this race 2 was found in several plant introductions from India, and from crosses involving them, several varieties with resistance to both forms of the mildew have been developed. Whitaker and Pryor (41) report that at least 2 or 3 genes seem to be involved in resistance to race 2 with resistance incompletely dominant.

Fusarium Wilt.—Work on resistance to *Fusarium oxysporum* f. *melonis* (Leach and Currence) Snyder and Hansen was begun in Minnesota following the appearance of that disease in 1931. Leach and Currence (23) discovered resistant plants in the varieties Honey Dew, Casaba, Persian and Honey Ball. From a chance hybrid between Honey Dew and Bender, Golden Gopher was selected and released about 1940. A fusarium resistant line from Minnesota similar to Golden Gopher was crossed with Bender in New York and from this cross the Iroquois variety was selected and released in 1943 (25). Work was continued at Cornell to get an earlier fusarium resistant variety. Iroquois was crossed with Delicious, and 4 backcrosses to Delicious were made, selecting for resistance after each one. By this procedure a resistant variety essentially identical to the parental strain of Delicious was obtained and named Delicious 51. It was multiplied by seedsmen in 1952.

The genic basis for fusarium resistance has not been definitely determined, in spite of the fact that it appears relatively simple. The presence of several other diseases make field counts of fusarium infection somewhat inaccurate, and greenhouse testing for resistance has never been entirely satisfactory. Young seedlings of resistant varieties transplanted to artificially infested soil in the greenhouse have died as quickly as susceptible varieties in experiments at Cornell, and McKeen (24) found that the supposedly resistant Iroquois died ever more quickly than a susceptible variety Hoodoo when direct seeded in infested soil in greenhouse flats.

After trying numerous methods of inoculation, Dolan (9) concluded that resistant and susceptible muskmelons could be differentiated best in the greenhouse by injecting a spore suspension of *F. oxysporum* f. *melonis* between the cotyledons of seedlings. Few susceptible plants survive this treatment, and most plants of resistant varieties remain healthy. Unfortunately, for studying the inheritance of resistance, some resistant plants develop symptoms of wilt and may even die following injections of spores. This percentage is not high but it varies with season, being higher in the winter.

Resistance has always appeared to be completely dominant in F_1 hybrids. F_2 and backcross data approach what one would expect if there were a single dominant gene for resistance, more closely than any other standard hypothesis. However, there have been significantly fewer susceptible plants than expected on this basis in several tests, with F_2 ratios of about 6 resistant to 1 susceptible being found frequently. In testing successive backcross progenies involved in breeding Delicious 51, inoculation by injecting spores was used almost entirely and there was no difficulty in maintaining the same level of resistance found in Iroquois. This confirms the idea that resistance is completely dominant and simply inherited. However, the proportion of F_3 and F_4 progenies showing segregation for fusarium resistance has consistently been larger than one would expect if only 1 gene were involved.

The death of resistant seedlings in infested soil in the greenhouse not only complicates studies of inheritance but also raises the question as to whether a variety which is resistant when transplanted at 3–4 weeks of age to an infested field may not be susceptible when direct seeded on infested soil. McKeen (24) cautions that Iroquois may show losses from fusarium wilt when direct seeded because of susceptibility in the seedling state, but he bases this on greenhouse tests rather than an observation of susceptibility in the field. Not many muskmelons are direct-seeded in New York State but 1 such planting of Iroquois has been observed on a field known to be heavily infested with fusarium. Here there was no evidence that the seedlings were susceptible.

Downy Mildew.—A considerable number of muskmelons have been found resistant to downy mildew caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow. Between 1935 and 1940, several from the West Indies were introduced directly as commercial varieties for the southeastern states where downy mildew is most severe. These include Cuban Castillian, Orange Fleshed Rocky Dew, Green Fleshed Rocky Dew, and Smith's Perfect. Ivanoff (17) reported

Smith's Perfect as the most resistant in Texas and the best horticulturally, whereas Weber (39) stated that in Florida, Cuban Castillian and Orange Fleshed Rocky Dew were most resistant. Texas Resistant No. 1 was developed by Ivanoff from a cross between Cuban Castillian and a strain of Hales Best.

More recently several melons with higher resistance to downy mildew but with poorer fruit qualities have been found, mostly among foreign plant introductions. Georgia 47 has been developed by van Haltern (37) from the cross (Hearts of Gold × P I 29,554) × Smith's Perfect. This has resistance to powdery mildew as well as downy mildew along with good flesh quality. Godfrey (15) has used a wild "smell melon" (*Cucumis melo* var. *chito*) as a source of resistance to downy mildew. By crossing it with Hales Best and backcrossing to Smith's Perfect he obtained Rio Sweet. Informal exchange of information among muskmelon breeders indicates that several other plant introductions are being used as sources of resistance but it appears that no one of these has immunity or a clear-cut advantage in resistance.

Ivanoff (17) reported that downy mildew resistance is partly dominant in the F_1 and that various degrees of resistance occur in later generations. Later he stated (18) that downy mildew resistance is closely associated with aphid resistance which appeared to be inherited as a single dominant factor. It is obvious now from the many degrees of resistance found in various introductions that a number of genes must be involved in downy mildew resistance.

Alternaria Leaf-spot.—Resistance to the leaf-spot disease caused by *Alternaria cucumerinum* (Ell. and Ev.) J. A. Elliott, is listed as an objective in several muskmelon breeding projects. Purdue 44 was introduced because of its alternaria resistance (16). It originated as a selection from the variety New Seed Breeders and there are genes for resistance in other commercial and foreign varieties. The inheritance of alternaria resistance has apparently not been determined.

Mosaic.—Mosaic resistance in muskmelons was first reported by Enzie in 1943 (13). He inoculated about 100 lots including several subspecies of *Cucumis melo* with cucumber virus 1 (*Marmor cucumeris* var. *vulgare*). Freeman Cucumber and White Melon which are horticultural varieties of *C. melo* var. *conomon* were the only lots which failed to show mosaic symptoms. Except for earliness, these melons have no desirable horticultural characteristics. Enzie initiated a breeding project for transferring resistance to edible muskmelons, and since 1943 that has been carried on at Ithaca, N. Y., by the senior author. Best results have been found where Freeman Cucumber was used as the resistant parent. That variety has been found in the field to develop a mild mosaic mottling which is scarcely discernible through much of the season. All gradations of resistance are found in its progenies indicating that there are a number of genes for mosaic resistance. Backcrossing has been essential to recover the characteristics needed in a commercial muskmelon variety and it has been difficult to retain a high degree of mosaic resistance. However, strains are now on hand which carry most of the original resistance and much improved type.

Bohn (5) and Bohn and Whitaker (6) have found tolerance to mosaic diseases which occur in California in both foreign plant introductions and in certain lines of powdery mildew resistant material.

Stem-Streak.—Dolan (10) described a new muskmelon disease which in certain years has been extremely destructive to muskmelons in New York. Pink or tan streaks appear on the stems, the tips of affected runners die at about the same time, and soon afterward the entire plant is dead. Dolan considered the causal fungus to be a species of *Marssonina* which he named *M. melonis*, but later work by Boothroyd (7) indicates that the correct name for the fungus is in doubt. We shall refer to the disease here as stem-streak.

Resistance to stem-streak was found by Dolan in the Freeman Cucumber (*C. melo* var. *conomon*) which had been used as a source of mosaic resistance and fully as much resistance was later found in progenies of Freeman Cucumber crossed with edible varieties. Many genes seem to be involved in stem-streak resistance since all gradations of resistance can be observed. Boothroyd (7) found that greenhouse testing is not very satisfactory as there is a rather narrow margin between an incubation period which brings about death of all plants and one which leaves susceptible melons without symptoms. However, good tests for resistance have been obtained in the field by spraying spore suspensions on the melon plants, and artificial inoculation has not been necessary in fields when stem-streak was severe the preceding season. Certain progenies have consistently remained alive and green and ripened their fruit long after commercial varieties have died with stem-streak before their fruit was mature.

CUCUMBERS.—*Mosaic.*—In cucumbers there has been extensive breeding for resistance to mosaic (Cucumber virus 1), downy mildew (*Pseudoperonospora cubensis*) and scab (*Cladiosporium cucumerinum*). The mosaic resistance of Chinese Long cucumber discovered in China by R. H. Porter in 1926 (12, 31) opened the way to the development of mosaic resistant varieties so badly needed in the northern United States. Shamrock, developed at Iowa State College, from a cross involving Chinese Long was the first mosaic resistant cucumber introduced for commercial use. Tokyo Long Green was later found by Doolittle et al (11) to have mosaic resistance, and it was the resistant parent for Ohio 31, a mosaic resistant pickling-type cucumber.

In breeding cucumbers at Cornell, we have observed a marked difference in mosaic symptom progression in Chinese Long as contrasted with Tokyo Long Green. Chinese Long develops a slight mottling in 10–14 days after inoculation and leaves which develop after that show progressively less mottling until no symptoms are evident. Virus cannot usually be recovered beyond

the fifth or sixth leaf from the point of inoculation. Porter (31) described the same reaction but stated that it occurred in only a few of the Chinese Long plants while the rest remained symptomless. We have found no symptomless plants and only 1 inbred line of Chinese Long which exhibits the complete recovery just described.

At 2 weeks after inoculation, Tokyo Long Green usually shows less distinct mottling than Chinese Long but it shows little tendency to recover and the symptoms usually become more pronounced as growth continues. The same contrast in symptom progression is readily seen by comparing Ohio 31, which gets its resistance from Tokyo Long Green, with Ohio MR 17 or Yorkstate Pickling, which get their resistance from Chinese Long.

Inheritance of resistance in Chinese Long × the susceptible Early Russian was studied by Shifriss, Myers, and Chupp (33) who concluded that there were 3 principal genes involved, all of which must be homozygous dominant in order for a plant to be as resistant as Chinese Long. In the F_2 they found a ratio of 27 plants with varying degrees of tolerance to 37 which were susceptible, as indicated by chlorosis of cotyledons followed by severe mottling and stunting. Under the uncontrolled field conditions of our breeding program, the inheritance of resistance appears much more complex than the report just mentioned would indicate, the reasons being as follows:

1) Occasionally it has been possible to disinguish the susceptible plants soon after inoculation as found by Shifriss et al, but more often all plants have shown symptoms and can be selected for resistance only after watching their recovery in the field through most of the season.

2) True breeding strains with all gradations of resistance are found, whereas under the hypothesis of Shifriss et al, the only resistant strains which are homozygous should be as resistant as Chinese Long.

3) Resistance to leaf mottling and to fruit mottling are independent to a disturbing extent.

4) Chinese Long does not remain symptomless as reported by Shifriss et al. The same inbred has been used in both cases but not the same source of virus so the latter may account for the difference.

Thus it is apparent that lack of immunity to mosaic greatly complicates a breeding program. It seemed that it might be possible to increase resistance or even produce immunity by combining genes from Chinese Long and Tokyo Long Green which show such different reactions to mosaic. We have grown F_4 progenies of the cross and results so far are encouraging. While it is not certain that we have attained immunity, entire progenies have been much more resistant than either parent and some individuals have not shown symptoms.

All of this has been to answer the question as to what source of mosaic resistance to use in a breeding program. We have favored the Chinese Long previously but think that some of the more resistant progenies (which we will gladly supply) should be considered for future work.

Downy Mildew.—Resistance to downy mildew has been found in several cucumbers from India (8) and China (21). China, Chinese Long, and Puerto Rico 37 (which apparently gets its resistance from Chinese Long) are the parents which have been most used as sources of resistance. Inheritance of resistance is clearly polygenic (8, 21). Immunity has not been found and this is a serious obstacle in a breeding program because several non-genetic factors affect the expression of resistance (4). Early maturity or a heavy fruit load tend to make a plant look more susceptible than one with the same genes for resistance but maturing later or with little fruit. Furthermore, evaluation of resistance is difficult because a resistant variety goes down more quickly in a planting which includes more susceptible varieties than it does in a solid planting, because of the difference in abundance of inoculum.

The varieties Palmetto and Santee (3) have shown striking advantages in the South although downy mildew suddenly became much more serious on Palmetto in 1950 (14) indicating a change in the causal fungus. In the years when downy mildew has been serious in New York, Palmetto has stood up only slightly longer than susceptible varieties while Puerto Rico 37 and Chinese Long showed much more resistance. It is obvious that not all of the genes for resistance have been transferred in breeding work to date because of the difficulties mentioned above. In New York we have grown F_2 progenies of Chinese Long × Tokyo Long Green and have had indications that resistance might be increased, but we have not gone far with this because downy mildew has not occurred every year recently.

Scab.—Resistance to scab caused by *Cladosporium cucumerinum* Ell. and Arth. was found by Bailey and Burgess (2) in individual plants of commercial varieties. Main 2 and Highmoor are scab resistant varieties resulting from their work. They found a single dominant gene responsible for resistance and the work of Walker (38) confirms this. This scab resistance can be transferred readily in breeding cucumbers.

Powdery Mildew.—Smith (34) discovered that Puerto Rico 37 has considerable resistance to powdery mildew and that multiple genetic factors are involved. No variety with powdery mildew resistance has been released in the United States as yet.

Squash.—Only 1 reference to disease resistance in squash has been found (36) which mentions incidentally that hybrids of *Cucurbita moschata* × *C. pepo* did not show mosaic symptoms while every *C. pepo* plant in the same field was severely affected. Van Eseltine initiated a breeding program to develop mosaic resistant *C. pepos* but no varieties were released from it. At least one of the varieties which Van Eseltine used as a resistant parent (Japanese Pie) is now classified as *Cucurbita mixta* (40).

Recently T. L. York and L. W. Schaible at Cornell have been attempting to find the best source of resistance for breeding mosaic resistant *C. pepos.* Most *C. moschatas* are resistant but some are not. The

great variation in resistance among the *C. pepos* seems worth investigating since there appears to be nothing published which indicates whether or not there is free recombination of genes between *C. moschata* and *C. pepo* even though the cross has been made many times.

DISCUSSION.—A great deal of effort has gone into breeding celery and cucurbits for disease resistance and many varieties have been released. It is difficult to determine from the literature the fate or the reasons for the fate of these varieties. However, a considerable number of them have not met the needs for which they were intended since after their release breeding programs with the same objectives have continued. Observation and a little published information indicate that resistant varieties more often prove unsatisfactory because of deficiencies in horticultural characteristics than because of lack of disease resistance. A breeding procedure which involves repeated backcrossing to a variety of proven commercial value until the genotype of the latter is recovered in combination with resistance would seem to be the ideal solution where it can be used. It would decrease rather than increase the number of varieties which seedsmen must offer, reduce the risk that a new variety resistant to one disease may be more susceptible to another, and permit growers to adopt disease resistant varieties after a shorter trial period.

Use of the backcross method is difficult where one starts with a high level of resistance rather than immunity, and inheritance is polygenic. As compared with simply inherited resistance, more generations must elapse between backcrosses and some genes for resistance are usually lost after each backcross. Because of this decline in resistance, backcrossing stops before the many desirable genes from the susceptible parent are recovered and the resulting variety lacks something from the horticultural standpoint.

Even though a highly resistant plant may survive and produce exactly as well as one which is immune, its expression of symptoms is subject to variation for non-genetic reasons and, therefore, it is much more difficult to retain high resistance when selecting after a cross is made. For this reason it seems advisable at the start of a breeding program to spend considerable time in searching for immunity among foreign plant introductions or to synthesize it by recombining genes from different varieties with partial resistance.

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METHODS OF DETECTING AND INVESTIGATING PLANT PARASITIC NEMATODES [1]

D. J. Raski

Nematologists have maintained for many years that plant parasitic nematodes constitute one of the most important problems in the agriculture of this country. This contention has been supported by mounting evidence of the great losses caused to growers by the more common species such as root-knot, sugar-beet, root-lesion, bulb and stem and citrus nematodes. In addition, as research has continued and our knowledge of the relationships of nematodes has been advanced, it is increasingly evident that there is a much broader range of activity of plant-parasitic nematodes and their influence on the production of successful crops. For one thing, there are many species of nematodes known to be plant parasitic whose ability to cause injury has not been thoroughly explored. The same applies to other species suspected as plant parasites by their associations in the soil and their relationships to other known parasitic species. The influence of nematodes on the final expression of other diseases also deserves much more attention.

The accumulation of information and data on the importance of plant-parasitic nematodes is a slow process. One reason has been the limited number of specialists trained in nematology who have been working in this field. Another factor has been the long established belief by many workers in agriculture that the only nematode problem of serious consequence is root-knot nematode. If an examination of the root system of suspected plants failed to disclose galls or swellings, it was not considered to be a nematode problem. This belief still persists in some areas even where other species of plant-parasitic nematodes are known to be present in great numbers and could account for unexplained responses following soil treatment by fumigation.

Another important reason for the slow progress in nematology is the soil-borne nature of these species of nematodes which places them in a very complex environment about which very little is known. This fact, plus their microscopic size, makes it difficult or impossible to demonstrate the presence of nematodes under field conditions. The detection of the various species of plant-parasitic nematodes can almost always be made by an examination of soil samples in the laboratory but this is at best a laborious and time-consuming task. There is no simple method of examination yet known that would make possible an adequate survey of a state or even a particular growing area to determine the presence and distribution of plant-parasitic nematodes. As a result the detection of nematode attacks on plants is usually made first on the basis of field symptoms and reports of poor growth or failures observed in the field.

FIELD DIAGNOSIS OF NEMATODE INFESTATIONS.—Unfortunately field symptoms as a rule are not specific for nematodes. Above ground, there may be reduced or unthrifty growth, dieback, wilting, reduction and discoloration of foliage, distortion of leaves, buds or stems, caused by nematodes but these may also result from other causes such as frost damage, low fertility, minor element deficiencies, sun burn, etc., or attacks by fungi, insects or other organisms. The same problem exists for the root system and other parts of plants in the soil. Galls or other distortions or swellings, necrotic lesions, mutilation or absence of fine feeder roots, may all be caused by nematodes but may also

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be the result of other pests, diseases or environmental conditions.

In a few cases nematode infestations can be demonstrated in the field. For example, galls of root-knot nematodes and females and cysts of the various species of *Heterodera* can be seen with the unaided eye. Active specimens of the bulb and stem nematode can also be observed with a hand lens. However, diagnoses made in the field must also be confirmed by laboratory examination. In the first place, there are many other organisms that can produce galls on the roots of plants including other species of nematodes as well as certain insects and bacteria. Besides, there are some cases in which root-knot nematode attacks do not produce galls on the roots of the plants. The designation by Chitwood (3) of several species of *Meloidogyne* with distinct host preferences has made the diagnosis of root-knot nematode attacks even more complex. Identification of the particular species involved is a difficult and precise examination which can only be accomplished in the laboratory.

The field diagnosis of even the common sugar-beet nematode, in California, at least, has been complicated by the recent discovery by Raski (11) of *Heterodera cruciferae* in commercial plantings of Brussels sprouts. This species very closely resembles the sugar-beet nematode but evidence to date shows it is limited in host range to plants belonging to the family Cruciferae. The host range of the sugar-beet nematode is very wide and includes many of the crucifers. Consequently the determination of the species involved is essential before effective control measures can be applied.

Detection by fumigation with chemicals.—The development of nematocides effective as fumigants during the past 8–10 years has proved to be invaluable in the research on nematode problems. Chemicals such as dichloropropene mixtures (D-D) and ethylene dibromide (EDB) are not generally considered to have significant fungicidal or bactericidal properties at the dosages ordinarily used. The application of these materials therefore is effective as a differential fumigation and as an indication, at least, of the possible role of nematodes in certain complexes or situations. The citrus nematode is a good example of the effective use of such fumigants. The importance of this nematode as the primary cause of the decline of many citrus orchards has been given greater acceptability in recent years on the basis of evidence by Baines and Clarke (2) which more clearly demonstrates the pathogenicity of the citrus nematode. But in addition, the recommended treatment of fumigation of replant sites as a successful control measure for the citrus nematode adds further evidence for the belief that the principal agent in these problems of citrus decline is the citrus nematode.

Another example of this method of approach is described in work recently reported by Nusbaum and Chaplin (9) on black shank of tobacco. The effectiveness of fumigation with ethylene dibromide in reducing the incidence of this disease in resistant varieties of tobacco definitely warrants further investigation of the presence of plant-parasitic nematodes and their role in the final expression of the disease.

The results of fumigation alone admittedly do not reveal the whole story, particularly in soil complexes involving more than 1 pathogen. It is essential that the interaction and relationships of nematodes and other organisms also be more thoroughly investigated and understood. But there is little doubt that nematodes alone, by tremendous numbers and their massive invasion and destruction of root tissues, can cause serious injury; in some instances no other pathogenic organisms have been isolated. Even in some of the disease complexes, nematodes are considered to be the primary pathogen, as for example, brown root rot of tobacco. From a practical standpoint such problems are essentially a question of nematode control. Many unsolved problems in agriculture today, identified by such poorly-defined symptoms as unthrifty growth or die-back and decline, could be further diagnosed by the use of fumigants to reveal possible nematode infestations as the source of trouble.

Even where some problems have been thought to be fairly well understood nematodes may be an important factor as, for example, recent investigations by Holdeman and Graham (8) concerning cotton wilt and resistance to it as affected by the sting nematode, *Belonolaimus gracilis* Steiner, 1949. They found that in the presence of the wilt fungus alone the resistant varieties showed 0 per cent and susceptible varieties 10 per cent incidence of disease; in combinations of the wilt fungus and nematodes, resistant varieties showed 52 per cent and susceptible varieties 88 per cent incidence of disease. The same picture has also been described by Young (16) with regard to tomato wilt and the decrease of resistance to it in the presence of root-knot nematode attacks.

Analysis of mineral elements in plant parts.—The analysis of plant material including leaves, stems, and roots for mineral content has also been an important field of nematode research. Conclusive evidence points to significant increases or decreases of many of the essential mineral elements, including sodium, potassium, phosphorus, magnesium, copper, and others, as a result of nematode attacks on the roots (4, 10, 12). This in part may assist in determining how nematodes affect plants but also is important in the detection and diagnosis of nematode problems. For instance, a diagnosis made on an analysis of the foliage alone can lead and has led to the erroneous conclusion that a particular situation was primarily a salt problem but actually was the result of a nematode infection.

Detection by use of host plants.—The use of host plant relationships is often used to demonstrate and even differentiate species of nematodes. However, there are many problems which make this study a complicated and confusing test. The differences in host plant preferences between different populations of root-knot nematode led to the description of several species of *Meloidogyne*. But work such as Allen (1)

has reported indicates that the complexity of these species is far from settled. In single egg mass studies he found much wider variations in morphological patterns than previously reported, and in populations of the same species from different cotton fields he found considerable differences in host plant reactions. The morphological separations are adequate to identify most material that has been collected. Yet there are some situations which cannot be explained solely on the basis of species differences. For example, there is an 80-acre field in Kern County, California, which was planted to alfalfa and cotton for 10 years or more. The year prior to planting peaches ⅓ of the field was planted to alfalfa and ⅔ to cotton. The next year Shalil peaches were planted and suffered severe damage on the portion previously planted to alfalfa. The peaches on the portion planted to cotton did fairly well. Some replants on S-37 rootstocks have also shown some damage. In this field alfalfa was known to be attacked only by *M. hapla*, cotton by *M. incognita* var. *acrita*. Recent samples taken from both the Shalil and S-37 peaches show only *M. javanica*.

Another example of this complex is in the cotton-alfalfa rotation commonly used in the southern San Joaquin Valley in California. In this case *M. hapla* on alfalfa and *M. incognita* var. *acrita* on cotton constitute a satisfactory answer to the successful rotation. However, in one case a field was treated with fumigants, then planted to cotton for 2 years. The following year alfalfa planted for the first time was injured on the untreated plots. This is contrary to the usual survival of the nematodes which go to alfalfa following cotton.

Soil sampling methods.—Nematode infestations can be qualitatively as well as quantitatively examined by the use of soil samples. Where a field, seed bed, or greenhouse bench is suspected of having a nematode infestation a composite sample should be taken from several places selected at random. Any areas known to be weak spots when the field is cultivated should also be included in the sample. After mixing the sample thoroughly a pint aliquot is adequate for examination. In control studies a 4-in. soil auger may be used and marked to take 6-in. or 1-ft. levels. The soil from each of these levels is mixed separately and a pint aliquot used to measure the survival of the nematodes. Some samples may be taken in profile sections, the method that was used by Thorne (15) to study the dispersion of fumigants. These samples are usually 2-in. cubes taken in a vertical cross section 14–18 in. wide and down to 18 in.

In any case soil samples can be washed and the nematodes counted or where *Heterodera* species are being studied cysts can be extracted and larval counts made after hatching from the cysts. Counts can also be made by growing susceptible plants in the soil samples for several weeks after which the roots are washed clean and examined for nematode development as in root-knot nematode galls or females of *Heterodera* species.

In the case of root-lesion nematodes, counts made from soil washings may be very low in number; adequate numbers for comparative purposes were obtained only by extracting the nematodes from weighed samples of root material. This can also be done for other nematodes such as spring dwarf nematodes in strawberry buds.

Laboratory examination of samples.—Soil samples are first soaked up in several pints of water in a pan, then washed through a series of screens. These screens should grade from 50–200 mesh and 3 are sufficient to recover the full range of various sized nematodes. These screenings are allowed to settle in a beaker for at least 15–20 minutes, then the excess water is poured off and the concentrate examined under the binocular microscope. It is essential that such microscopes be equipped with a substage and mirror to provide reflected light. Direct light is not suitable for this purpose.

This last technique often contains an excessive amount of fine soil particles that cannot be eliminated. To save time in examining such samples, the funnel technique described by Christie and Perry (5) can be used. Fine muslin is sewed to metal rings that fit inside a funnel which is stoppered by rubber tubing and a clamp. The sample is poured through the cloth and the water brought up to the cloth to keep the sample soaked. Within 24 hours most of the active nematodes will work through the cloth and settle to the bottom where they can be drawn off in a small volume of clear water.

A direct examination of plant material may be made in the laboratory under a binocular microscope by teasing apart portions of roots, stems, leaves or buds in a dish of water. Plant material may also be cut into small pieces and soaked in water for 24 hours. After that time the excess water is poured off and the remainder searched for nematodes under the microscope. It must be remembered in making examinations of this kind that many saprophytic species of nematodes may be present. This is particularly true where necrosis is very extensive and secondary organisms have advanced the decay of tissues.

The funnel technique is also very useful in quantitative tests for nematodes in root or leaf samples. Roots are first washed clean and cut into small pieces. Weighed samples are then placed in the muslin filters and the water level raised to cover it. Nematodes which emerge from the roots work through the muslin and settle to the bottom of the funnel where they may be drawn off and counted. Root-lesion nematodes studied in this way continued to emerge from roots over a period of more than 2 weeks. The nematodes must be drawn off and the water changed at least every other day to reduce the build-up of fungi and bacteria.

Staining plant tissues.—Fixing and staining nematodes in plant tissues as described by Goodey (7) is also a method commonly used to demonstrate these pests and is especially useful for root material. The

roots are first washed clean, then placed in boiling lactophenol containing acid fuchsin dye for 1–2 minutes. After rinsing in water the roots are best stored for several days in clear lactophenol. In some cases it is very difficult to dissect obese females, such as root-knot nematode, from the tissues of fresh material without rupturing the body. Staining with acid fuchsin in lactophenol makes it possible to find the nematodes more readily and the plant tissues thus softened are separated more easily with a minimum of damage to the nematodes.

PREPARATION OF TEMPORARY MOUNTS.—Fresh nematode specimens can be examined quickly under the compound microscope by transferring the specimens to a drop of water on a slide. A short sliver of bamboo tapered to a fine point is best for handling them. This enables examination under the high dry objective. Active forms may have to be slowed down or killed in the drop of water by gentle heat. Glass supports may be placed in the water to avoid crushing the specimens and a coverglass dropped in place. These supports must be approximately the diameter of the specimens and can be made by heating glass rods and drawing them out to fine threads. Glass wool can be used for some of the smaller specimens. The coverglass is then secured by hot wax from a heated candle. Specimens mounted in this manner can be observed under oil immersion and will last for several hours or even overnight if stored in the refrigerator.

PRESERVATION AND PERMANENT MOUNTING METHODS.—Fresh specimens are best fixed for permanent mounts by killing them with gentle heat in a small quantity of water (125° F. for 5 minutes). The water is then drawn off and the following schedule followed:

1) Fix in F.A.A. fixative for 24–72 hours (95 per cent alcohol—20 parts; glacial acetic acid—1 part; formalin—6 parts; distilled water—40 parts).

2) Transfer to 2½ per cent glycerine in 30 per cent alcohol for 24 hours.

3) Transfer to 5 per cent glycerine in 30 per cent alcohol and dehydrate slowly for 1–2 weeks.

4) Place in desiccator for 48–72 hours and mount in dehydrated glycerine.

A cavity slide of about 0.5 ml. capacity is best in Steps 1 and 2 and the cavity closed with a square coverglass. Solutions can be changed with the use of a small dropper drawn to a fine point. This should be done under a dissecting microscope to avoid losing specimens. In Step 3 it is best to use a 1 in. syracuse dish and fill to capacity so that after dehydration sufficient glycerine will remain to cover the specimens. Glass supports are again necessary to prevent flattening of the specimens and Thorne's ringing compound (14) ensures a permanent mount that can be kept indefinitely. The principal disadvantage to this method is that it takes a minimum of 8–10 days to get a collection mounted. However, this is the most satisfactory method of fixation and causes the least distortion of specimens of all the methods so far tested.

Mass collections of unmounted nematodes are often made after representative specimens have been secured for mounting. The nematodes in the remainder of the washings are killed with boiling water and allowed to settle. The excess water is then poured off and an equal amount of 10 per cent formalin added. It is essential that this procedure be followed because cold fixative usually distorts the specimens excessively. This is a very simple and effective way of preserving material for future reference and identification and could readily be adopted as a measure of ensuring an evaluation of nematode populations that could possibly influence other investigations.

It is desirable in some cases to fix specimens in lactophenol for a more rapid examination. The specimens are killed by gentle heat in water then transferred to a drop of lactophenol slightly tinted with acid fuchsin. This is heated gently for not more than 1 minute until the specimens are cleared; then they are transferred to cold lactophenol in which they are mounted. This is a drastic treatment, however, that makes internal characteristics difficult to see and often distorts the spear but makes certain cuticular characteristics more pronounced. This method was adapted from Franklin and Goodey (6) but is recommended only as a supplement to the other fixation process although these mounts can also be kept permanently.

The importance of making permanent collections whether mounted or in mass collections cannot be emphasized strongly enough. Actually it is a standard procedure used in most branches of science but as our knowledge of nematodes is increased it is becoming more and more essential that reference material be made available. The taxonomic status of many common forms is still very unstable. In the case of *Pratylenchus pratensis* there are many publications on important work on biology and control which have little or no value in view of the present concepts of this genus. There are at least 10 species of the genus *Pratylenchus* that can be recognized today and many more undoubtedly exist. Much of the work done in the past has been published under the name *pratensis* and wherever specimens are not available for positive identification there will always remain some doubt as to the species being studied. In one instance there were no less than 3 different species of root-lesion nematodes present in a field that was used for extensive studies yet were recognized as only 1 species.

The genus *Pratylenchus* is further complicated by lack of information on at least 1 species which is frequently encountered in samples collected in California. So far it has not been possible to determine its feeding habits and it has never been found infesting roots examined along with the soil samples. In view of this, the determination of the presence of root-lesion nematode is not sufficient evidence of an economic infestation. A specific determination must be made to confirm root-lesion nematode attacks.

Host plant studies, rotation programs, resistant rootstock trials and control tests can not be correctly evaluated or properly understood unless the nematode

populations which are being investigated are identified by specialists trained in nematology. Permanent collections should be made for future identification in any situation where nematodes are known to be a factor or are even suspected of influencing results. It would also be desirable and is recommended that such collections be deposited someplace where a nematode collection is being maintained.

SUMMARY

The detection of some nematode infestations of cultivated crops can be made under field conditions. Usually such diagnoses must be confirmed by laboratory examination and identification of species. Soil fumigation with nematocides such as dichloropropene mixtures (D-D) and ethylene dibromide (EDB) is useful as an indication of nematode presence or influence in many plant disease situations. Increases or decreases of mineral elements in plant parts may also be an indication of nematode attacks on the root system. Host plants can be used to detect and even differentiate some species of nematodes but is a complex and sometimes inconclusive test. Soil sampling techniques are described as well as methods of examining samples of soil or plant parts in the laboratory. A binocular microscope equipped with a substage and mirror to provide reflected light is essential in this work. The process of fixing and preserving specimens and methods for making temporary and permanent mounts are discussed. The procedure for making mass collections of unmounted nematodes is also described. It is urged that this simple and effective technique be widely adopted as a permanent means of evaluating possible nematode influences in agricultural investigations.

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METHODS OF ASSAYING NEMATICIDES [1]

C. W. McBeth and G. B. Bergeson

Until the discovery of D-D[2] (Dichloropropene-dichloropropane mixture) and ethylene dibromide, the cost of soil fumigation on a field scale for nematode control was prohibitive except for crops of very high cash return, such as are grown in nurseries and greenhouses; consequently, there was no great interest in the development of new soil fumigants. After the first publications on the effectiveness of D-D by Carter (2) in 1943, and of EDB by Christie (3) in 1945 the interest in the development of soil fumigants increased, resulting in greatly accelerated research both by chemical companies and by state and federal workers.

The preliminary or primary screening work is being conducted, in most cases, by commercial laboratories because they have access to greater numbers and wider varieties of materials. The secondary screening is conducted by commercial, federal and state workers, both individually and cooperatively.

Both commercial and other research workers have their own special techniques for screening chemicals for nematicidal activity, these methods being designed to meet their own specific needs and interests. No attempt will be made to discuss the various methods of assaying chemicals in use by various workers, but some general consideration applicable in selecting procedure for testing materials will be discussed together with the methods in use by the authors.

For setting up a nematicidal screen it must be recognized that chemicals currently used for nematode control are largely fumigants: that is, they travel from the point of application to the nematode in the vapor phase. Volatility then becomes an extremely important factor in testing nematicides. However, other methods of application are feasible, such as by emulsion, rototiller, disk, etc.; these do not depend entirely on volatility for dispersion. If mixing is inadequate, the toxicity of a material may be obscured by lack of volatility. Certain materials are highly sorbed by the soil particles which may obscure basic toxicity also. The primary screen, then, must be designed to test toxicity independently of the soil in order to eliminate such factors as volatility and sorption or inactivation of the chemical by the soil. Because most nematodes require a film of moisture for survival and a nematicide must penetrate this film to come in contact with the nematode, the most practical method of testing chemicals for basic toxicity is in an aqueous solution or emulsion. The data from such a primary screen, together with preliminary knowledge of such physical properties as boiling point, will serve as a basis for deciding the type of secondary screen to be used.

[1] An invitational paper presented at the Symposium on Plant Parasitic Nematodes at the 44th Annual Meeting of The American Phytopathological Society in Ithaca, N. Y., September 8, 1952.

[2] D-D, a soil fumigant, Trade Mark Reg. U. S. Pat. Off.

Having selected chemicals of known toxicity to at least 1 species of nematode, secondary screens involving the use of soil may then be used. With highly volatile materials such as methyl bromide, it is necessary to confine the fumes in the soil by some artificial means. For this purpose sealed containers, such as glass fruit jars, are satisfactory and eliminate the danger of discarding highly volatile materials as ineffective. If one considers that general field application precludes the use of soil seals, a screen based on injection into a simple open container is desirable. One-gallon glazed crocks are satisfactory for this purpose as they eliminate the problem of loss of fumigation through porous containers such as clay pots; they are also satisfactory as containers for constant temperature water baths.

For best results all of these tests must be conducted under controlled temperature conditions and preferably at 2 or more temperatures, especially in the soil injection technique.

Apparently the species of nematode to be used as a screening organism is not too important as specificity of chemicals is negligible. By this, it should not be construed that all nematodes are equally susceptible to chemical control. It is known that cyst-forming nematodes are less susceptible to chemical control than certain other non-cyst-forming species but this is primarily due to the stage in the life cycle at which the treatment takes place, namely, the egg stage, plus a certain amount of protection by the cyst coat. Certain other forms appear to be somewhat less susceptible to fumigants due to a tougher cuticle. Other species appear to be more or less susceptible depending on the time of year or age of the individual. There is no indication, at present, that nematicidal specificity is important in screening work but as a matter of safety, it is felt advisable to use economically important, plant parasitic nematodes as test organisms.

In all nematicidal screening work a chemical of known toxicity, preferably a material of proven commercial value such as D-D (dichloropropene-dichloropropane mixture) should be included along with an untreated check. Because it is so difficult to maintain all controlled conditions constant from test to test, it is advisable to include each chemical at a minimum of 3 dosage levels with each dosage replicated 2–3 times. Questionable or inconclusive results are very wasteful in time, material and expense.

SCREENING TECHNIQUES IN USE BY THE AUTHORS.—1) *Water screen.*—In this screen the test chemicals are dissolved or emulsified in water, depending on the solubility, at concentrations dependent on the effectiveness of the standard check material (D-D) to the particular test nematode used. The bulb and stem nematode, *Ditylenchus dipsaci*, Kuhn, is particularly suitable as it is a relatively active plant parasitic nema-

tode which makes mortality counts much simpler than with the inactive root knot larvae. Also, this species can be kept in a dormant condition (nematode wool) without special care or equipment; thus a ready supply of a pure culture of living nematodes is available at all times. As D-D is used as a standard material and approximately 400 ppm is required for 100 per cent mortality of this nematode, the dosages used are 200, 400 and 800 ppm. This divides the effective materials into those equal to D-D, those approximately half as effective, and those at least twice as effective as D-D. Those not effective at twice the effective dosage of D-D are eliminated as ineffective. This test is conducted in sealed hollow ground slides at a constant temperature of 75°F., the nematodes being left in contact with the chemical for 24 hours. The water screen technique is not infallible and should not be depended upon altogether as certain compounds highly effective by injection, especially volatile materials, have failed to show a high degree of toxicity by this method of testing.

2) *Soil injection screen.*—Following tests in the primary water screen the effective chemicals must be added to the soil to determine if the nematicidal effectiveness is maintained or nullified by the soil. For volatile materials, the most effective method of testing is to inject test chemicals directly into soil naturally infested with plant parasitic nematodes. Due to effects of soil types on effectiveness of soil fumigants (1), it is advisable to use a standard soil type for all tests. The root knot nematode (*Meloidogyne sp.*) lends itself to this method of testing, since an indicator crop such as tomatoes can be very easily grown in the treated soil and the degree of knotting or galling can be used as an index of the nematicidal activity of the material.

From the standpoint of a commercial laboratory, with a large number of materials available, it is necessary to devise a method whereby a large number of materials can be tested under conditions favoring the least effective materials. In this case the ideal screen eliminates all ineffective materials but retains those which possess any measurable nematicidal qualities. For this purpose small sealed containers of soil (1 or 2 qt. fruit jars) have proven to be most practical for use. The test should be conducted under as nearly constant and reproducible conditions as possible. From our experience it is desirable to conduct tests in controlled temperature boxes or rooms at at least 2 constant temperature levels (60° and 80°F.). If only 1 temperature is used, it should be the higher in order to reveal effectiveness of higher boiling point materials; effectiveness of low boiling point materials would be revealed at either temperature since they are enclosed in a sealed container.

Materials that are nematicidal in this preliminary test are then injected into open containers of nematode infested soil (1 gal. glazed crocks are satisfactory for this). The exposure time should be at least 1 week for both jar and crock tests. In the open crock test, also, soil temperature, moisture and soil type should be kept as nearly constant as possible and at least 3 temperatures should be used in order to determine the optimum for a given chemical. In general, chemicals with boiling points over 150°C. require high soil temperatures (75–80°F.) for effectiveness; lower boiling point materials (100°C.), such as D-D, are effective as low as 45°F. as well as at high temperatures. If only a small number of materials are to be tested, the primary or sealed container phase of this screen can be eliminated. Approximately 6 weeks is required for completion of this type of test. Field testing is not advisable until the open container screen has been conducted because of the amount of time and material required. Also, field conditions are so variable and unpredictable that too much time is lost in determining the ideal conditions for a material.

3) *Soil mix screen.*—Because many materials which can be effective in the water screen can also be relatively non-volatile, the injection technique is not universally useful. Two other methods are used by us: 1) the water drench, and 2) mixing the chemical intimately with the soil. Both techniques have their merits and are desirable, but if only 1 is to be used, it has been found that the soil-mix technique is to be preferred since it will reveal any effective material which can be demonstrated by the drench method, whereas the latter technique will not always reveal materials shown to be effective by the soil-mix. The soil-mix screen is conducted as follows: the chemical is impregnated on talc or some other carrier. The impregnated carrier is then mixed thoroughly with naturally infested soil using a mechanical mixer (such as the "Twin Shell Mixer," manufactured by Patterson Kelley Company, Stroudburg, Pennsylvania) or by rolling and tumbling in a sealed container by hand. Either method is effective but the mechanical mixer is more dependable as there is less variation in degree of mixing. When the chemical is well mixed with the soil which requires approximately 1 minute, the treated soil is potted and seeded to tomatoes as an indicator crop.

There are other methods of determining the nematode kill in the treated soil based on actual nematode counts. These require less elapsed time, but require more time of the operator. The first is a method described by Cobb (5) which is a method of separating the nematodes from the soil by use of fine mesh screens. The main drawback to this method is the amount of operator time required and the difficulty of determining accurately if the nematodes are dead; it is particularly difficult with the root knot nematode because it is so inactive. The second method is an adaptation of the Baerman funnel as described by Christie (4). This method also requires considerable time and it is not especially dependable for our purpose. Our experience has been that certain chemicals temporarily inactivate the nematodes so that they do not emerge by the funnel, but are found to be alive when an indicator crop is planted in the soil.

4) *Field testing.*—Materials which have consistent-

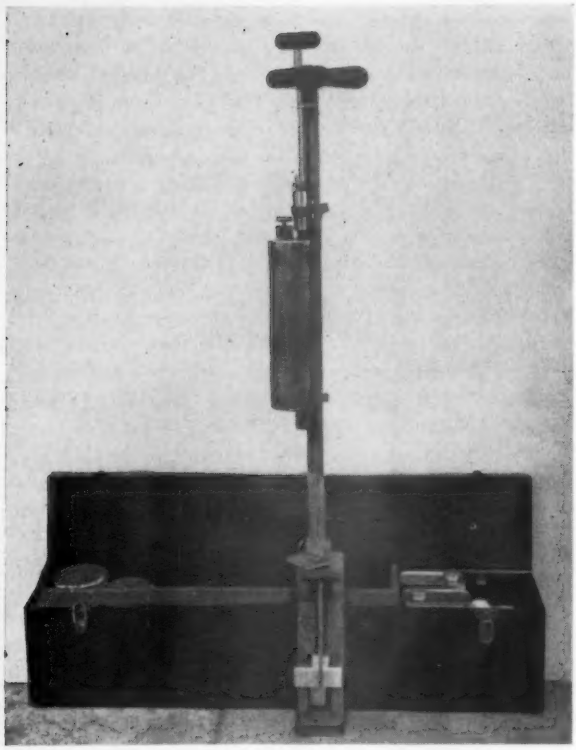

FIG. 1. Precision built hand injector and carrying case.

ly appeared promising by the above series of laboratory and greenhouse tests and are economically attractive are taken into the field for final assessment. The method of testing depends as before upon the physical nature of the material. Irrespective of the type of material to be tested, the main problem is to make certain the test area or field is sufficiently and uniformly infested with the desired species of nematode. The soil type and tilth should also be uniform. For volatile materials our technique is as follows. Each chemical is tested at at least 3 dosage rates, at the calculated effective dosage (from crock test), at double, and at ½ this value. By this means a close approximation of the actual effective dosage under field conditions can be determined. Naturally, a commercial material is included in the test at known effective dosages. Each dosage of all chemicals is replicated at least 3 times and preferably more. The test is designed in such a manner as to make statistical analysis possible. A randomized block design or latin square is very satisfactory for this work.

After it has been established that the nematode population, soil type, soil tilth, temperature and moisture conditions are satisfactory, the test area is staked out into the desired number of plots. It has been found that 8 ft. × 8 ft. sq. plots are large enough, providing the plot design is satisfactory and irrigation can be accomplished by an overhead system to reduce the chances of contamination from plot to plot.

The results obtained from an injection treatment can be no more dependable than the equipment used. A very accurate and dependable hand injector has been designed and built at the Shell Agricultural Research Laboratory based on a model designed by A. L. Taylor, U.S.D.A. Nematologist. A photograph of the injector and case is included in Fig. 1. Construction details are availiable upon request. This injector can be calibrated from 0.5 ml. per shot to 3 ml. At present this injection kit is being manufactured by the Whitey Tool and Die Company, Oakland, California.

The spacing and location of injection points is very important in this type of test. The most efficient spacing is at the apices of equilateral triangles as described by Taylor (6). A triangular injection spacing of 13.9 in. is very convenient for calculating dosage as 1 ml. per injection is equal to 10 gal. per acre. For locating the injection points, the plot is marked off by means of a rake type row marker as shown in Fig. 2. The rake or marker has 2 sets of pegs at right angles to each other. The pegs on 1 side are 12 in. apart and on the other 13.9 in. The 12 in. spacing is used for marking the plots in 1 direction and the 13.9 in. spacing at right angles. The result is a grid of 12 in. × 13.9 in. rectangles. The injection points are located on the lines 12 in. apart alternating between the intersections of the 13.9 in. lines and half way between the intersections which results in the equilateral triangle arrangement.

Plantings are made at intervals after treatment for phytotoxicity readings or after a 2-week delay as an indicator for nematicidal readings. Yellow crook

FIG. 2. Plot being marked for spacing injection points.

neck squash is satisfactory as an indicator crop as it is easy to grow and the roots are satisfactory for revealing even a very light nematode infection. The plantings should be made in rows 12 in. apart in order to have at least 100 plants from each plot for examination. Indicator plants are not taken within 1 ft. of the edge of the plot to eliminate border effects. The plants are dug carefully and examined visually for knotting or galling; either per cent infection or root knot index can be used as a measure of the nematicidal toxicity of the material. Although the results are usually well defined, it is advisable to analyze the results statistically in order to be sure that the differences are real and not due to chance.

If more information on the dispersion pattern of the material in the soil is desired, soil profile samples can be taken in the manner described by Thorne (7).

For field testing materials as aqueous drenches, a metal frame approximately 2 ft. in diameter is used to confine the emulsion in the test area. Because of the importance of depth of penetration of the chemical, heavily nematode infested soil is worked into the area to be treated to a depth of 1 ft. in order to assure a well distributed population to that depth. One week after the application has been made, the treated soil is taken up in 1-in. layers down to 12 in. deep and each layer potted separately. The potted soil is planted to tomatoes as an indicator. In this manner the depth of penetration of the material can be determined very accurately. Some materials which are exceedingly effective in the primary water screen are only effective in the surface inch or two due to sorption by the soil, while others such as CBP-55 (chlorobromopropene) and D-D are effective to the full 12 in. depth.

Mechanical means for applying chemicals that require thorough mixing with the soil are only in experimental stages. Fair control has resulted experimentally with rototiller and disk harrow applications, although no materials of outstanding effectiveness are being used commercially by the method.

SUMMARY

Due to wide variations in physical and chemical properties of compounds, there is no single screening technique that will separate all of the nematicidal from the non-nematicidal materials. The following screening methods have been adopted by our laboratory as an assurance that no effective materials will be discarded.

1) *Water Screen.* Chemicals are tested in aqueous solution or emulsion for basic or inherent toxicity. The bulb and stem nematode, *Ditylenchus dipsaci*, Kuhn, is used as a test organism.

2) *Soil Injection Screen.* This and the following procedures are used to determine the effect of soil upon the nematicidal value of the material. Volatile materials are injected into either sealed or open containers of root knot nematode infested soil, depending on the volatility of the material. Effectiveness is measured by an indicator plant such as tomato.

3) *Soil Mix Screen.* Relatively non-volatile materials are impregnated upon a solid carrier and mixed intimately with root knot infested soil. Tomatoes are used as an indicator of the effectiveness of the material.

4) Materials which merit further consideration from a nematicidal and an economic viewpoint are tested under field conditions in small replicated plots before being released to other research workers.

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THE EFFECT OF CONTINUAL USE OF CERTAIN FUNGICIDES ON PHYSALOSPORA OBTUSA [1]

Jack Taylor

Fungicides in common use in the mountains of Georgia have been of little value in controlling apple black rot (*Physalospora obtusa* (Schw.) Cooke) on the fruit (3), nor have they been entirely satisfactory in controlling leaf symptoms. During 1951 about 62 per cent of all apple drops were affected with black rot. Infection varied with varieties. About 73 per cent of Golden Delicious, 60 per cent of Red Delicious, and 30 per cent of Stayman Winesap drops showed this disease. In some instances more than 75 per cent of the fruit set dropped before harvest time. This suggested the possibility that fungicides used in certain orchards for a long period of time have resulted in selection of resistant strains of the organism. To test this hypothesis, several fungicides were tested against conidia from different sources.

MATERIALS AND METHODS.—With slight modifications the slide-germination method of evaluating fungicides proposed by a committee of the American Phytopathological Society (1) was followed.

Slide toxicity tests were made on January 13 and 19, February 15, and March 27, 1952. On these dates pycnidia from cankered apple twig collections contained mature conidia. The sources of spores described in Table 1 consisted of orchards that had been sprayed with wettable sulfur, or lime-sulfur, until about 20–30 days after the blossoming period. Bordeaux mixture was used in subsequent sprays. Some of the material was collected from unsprayed home orchards.

Pycnidia were removed from the twigs and a distilled water spore suspension was made to contain 200–300 spores per drop.

Five wax pencil rings were made on each slide, and the slides were then sprayed under 15 lb. pressure with a horizontal air compressor and spray gun at a distance of 4 ft., using 2.4 gm. $CuSO_4$ and 4.8 gm. $Ca(OH)_2$/l.; 9.6 gm. $CuSO_4$ and 9.6 gm. $Ca(OH)_2$/l.; 9.6 gm. sulfur/l.; 1.8 gm. ferbam[2]/l.; 1.8 gm. ziram[3]/l.; 1.8 gm. MEBD[4]/l.; and 10 cc. lime-sulfur/800 cc. One slide with the 5 replications was used for each treatment and each source of spores. The spray was allowed to dry before a drop of the spore suspension was added to each ring. Slides were then placed in a moist chamber at 25°C. for 24 hours before germination counts were made.

One hundred spores were counted in each of the 5 replications on each slide. This permitted 500 spores to be observed for each source and for each treatment.

RESULTS.—The discrepancy between sources of the fungus and fungicides tested is shown by the significant interaction in the January and February tests (Table 3). Spore inhibition by Bordeaux mixture A (2-4-100) varied somewhat according to source except in the March test, but showed a significant reduction of spore germination in all tests. Bordeaux mixture B (8-8-100) was almost invariably more toxic to the organism than Bordeaux mixture A.

Good spore germination and hyphal growth took place on all untreated slides (Tables 2 and 3 and, Figures 1, A).

Sulfur and lime-sulfur did not inhibit spore germination, and mycelial growth was vigorous and branched profusely (Figure 1, C). Ferbam gave a germination range of 6-88.2, but spore inhibition was significantly

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[2] Ferric dimethyl dithiocarbamate.
[3] Zinc dimethyl dithiocarbamate.
[4] Manganese ethylene bisdithiocarbamate.

TABLE 1.—*Source of organism*

Location	Symbol	Approximate seasons sprayed	Commercial or home orchard	Principal materials used
Fannin County	I	8	commercial	lime-sulfur Bordeaux
Gilmer County	II	30	commercial	lime-sulfur Bordeaux
Habersham County	III	25	commercial	lime-sulfur wettable-sulfur Bordeaux
Union County	IV	10	Mt. Station	lime-sulfur Bordeaux
White County	V	—	home	none
Gilmer County	VI	—	home	none
Rabun County	VII	—	home	none
Clarke County	VIII	5	U. of Ga. Hort. farm	lime-sulfur Bordeaux

TABLE 2.—*Germinated P. obtusa conidia on treated slides after 24 and 48 hours at 25° C. from source VIII*[a]*, January 13, 1952*

	Check	Bordeaux A[b]	Lime-sulfur	Magnetic sulfur	Ferbam	Ziram	MEBD
	means[c]	means	means	means	means	means	means
24 hr.	94.0	25.8	80.6	95.8	34.8	54.6	0.0
48 hr.	94.2	25.0	83.4	92.4	54.6	69.4	0.0

[a] Symbols represent locations as described in Table 1.
[b] Bordeaux mixture B was not included in this test.
[c] LSD for treatment = 13.8; difference between 24 and 48 hour observations not significant.

better than the checks. Ziram showed similar effects on the organism in the tests where it was included. These materials exhibited a fungistatic effect on mycelial growth. Hyphae of the germinated spores attained lengths of 20-100μ (Figure 1, B) but failed to develop further. Table 2 shows germination readings at 24 and 48 hours; no change was observed after 72 hours. The MEBD fungicide and Bordeaux mixture B had a toxic effect on spore germination in every test.

DISCUSSION.—Results of January and February tests indicate that the weaker Bordeaux mixture would give satisfactory control of diseases caused by *P. obtusa* in the unsprayed home orchard, but would not effect control in some commercial orchards where that material has been used over a long period of time. This agrees with results obtained in Fannin and Gilmer county orchards in which 2-4-100 Bordeaux mixture failed to control either leaf spot or fruit rot in seasons when that material was used during an infection period. The variation of resistance to Bordeaux mixture A exhibited by conidia from different commercial orchards suggests that the continual use of that fungicide will not necessarily cause resistant strains to develop in any given length of time, but the data show that resistance may evolve after the use of Bordeaux mixture for only 8 years.

According to Walton (4) 80 per cent of the leaf spot infection usually occurs from the beginning of the blossoming period until 2½ weeks after petal fall. This is the period in which sulfur or lime-sulfur is the

TABLE 3.—*Germinated P. obtusa conidia on treated slides after 24 hours at 25° C. on 3 dates*

Source	Check	Bordeaux A	Bordeaux B	Lime-sulfur	Magnetic sulfur	Ferbam	MEBD	Source
1/19/52[a]	means	means	means	means	means	means	means	grand means
I	93.4	59.8	0.0	90.0	97.6	88.2	0.0	61.3
II	97.2	33.2	0.0	68.0	88.0	13.4	0.0	42.8
III	93.8	3.0	0.0	84.0	91.8	67.0	0.0	48.5
IV	98.4	1.2	0.0	96.0	81.4	72.2	0.0	49.9
V	96.2	12.4	0.0	73.4	92.2	56.8	0.0	47.3
VI	97.6	1.6	0.0	84.8	88.6	86.4	0.0	51.3
Treatment grand means	96.1	18.5	0.0	82.7	89.9	64.0	0.0	
2/15/52[b]								
I	78.4	65.0	0.0	87.8	72.0	60.2	0.0	51.9
II	86.8	35.0	0.0	55.6	78.8	27.4	0.0	40.5
III	97.7	3.6	0.0	98.2	95.0	54.0	0.0	49.8
IV	88.8	8.6	0.0	56.0	59.0	36.0	0.0	35.5
VI	83.8	0.8	0.0	76.0	94.2	17.4	0.0	38.9
VII	82.4	0.6	0.0	53.4	74.4	8.2	0.0	31.3
Treatment grand means	86.3	18.9	0.0	71.2	78.9	33.9	0.0	
3/27/52[c]								
I	90.0	1.4	0.0	89.4	87.8	6.0	0.0	39.2
II	94.6	4.2	0.0	65.6	81.8	6.2	0.0	36.1
III	80.2	1.6	0.0	71.2	90.6	12.0	0.0	36.5
IV	83.0	0.0	0.0	73.2	83.8	11.2	0.0	35.9
V	84.6	0.0	0.0	68.8	72.8	14.8	0.0	34.4
Treatment grand means	86.4	1.4	0.0	73.6	83.3	10.0	0.0	

[a] LSD for treatment = 20.4; source = 12.1. Interaction: calculated F = 2.09; tabulated F = 1.72.
[b] LSD for treatment = 15.2; source = 10.6. Interaction: calculated F = 8.80; tabulated F = 1.61.
[c] LSD for treatment = 13.5; source and interaction differences not significant.

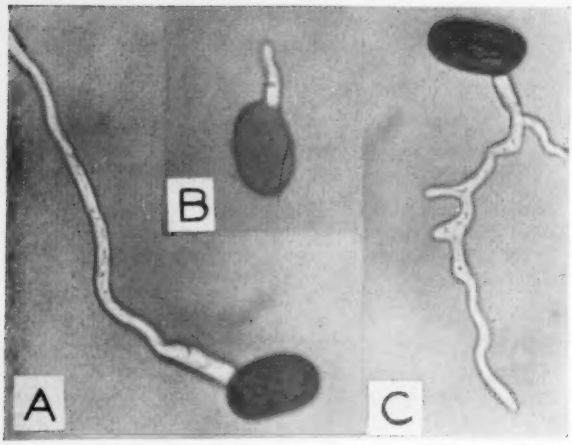

FIG. 1. *P. obtusa* conidia showing the effect of fungicides on germination and growth, A) spore germinated on untreated slide; B) spore germinated on slide treated with ferbam; C) spore germinated on slide treated with sulfur.

only fungicide recommended in the spray schedules of most southeastern states. Spraying experiments by Wolf (5) in northern Alabama in 1912, and Walton in Pennsylvania in 1920 showed that lime-sulfur was not effective against the black rot organism. Others have reported control of leaf spot when using lime-sulfur during the period in which the leaves are most susceptible to infection (2), but they failed to report weather records in connection with their experiments. Where ferbam has been used in the orchard spray schedule it has given relatively good control of leaf spot but has failed to control black rot on the fruit. The MEBD fungicide has given good control of leaf spot in field experiments and significant reduction of fruit infections.

The greater inhibitory effect of Bordeaux mixture A and ferbam on the spores collected in March probably explains the lack of interaction and the lack of difference between sources.

SUMMARY

Physalospora obtusa conidia from different sources were used in a slide toxicity study against several fungicides. A significant interaction between treatment and source of spores occurred in 2 tests indicating that the source of organism affected treatment results. This was noted especially in the 2-4-100 Bordeaux mixture. There was a tendency for that material to show greater inhibition to spores from orchards that had not been sprayed than to spores from orchards in which Bordeaux mixture had been used over a long period of time.

Manganese ethylene bisdithiocarbamate, an experimental fungicide, and 8-8-100 Bordeaux mixture almost completely inhibited germination of conidia in the laboratory. Ferbam and ziram inhibited germination of more than 50 per cent of the spores observed, and germination was significantly less than on the untreated slides. These materials stopped growth of germ tubes at a maximum length of 80-100μ. Inhibition of spore germination by wettable sulfur and lime-sulfur was not significant.

The milder Bordeaux mixture and ferbam proved more effective on conidia collected and tested in March than in January and February.

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THE MOSAIC VIRUS DISEASE COMPLEX OF LADINO CLOVER [1]

Byron R. Houston and John W. Oswald [2]

The Ladino variety of white clover (*Trifolium repens* L.) grown extensively throughout California has exhibited severe damage by what appears to be a number of virus diseases. Symptoms vary from a very mild chlorotic mottle to severe distortion and stunting accompanied by a marked mosaic pattern. Affected plants may sometimes be scattered in a planting, but more often nearly all plants show symptoms. Investigations were undertaken to determine the important and common viruses present in Ladino clover.

LITERATURE.—Transmissions of viruses from naturally infected white clover have been reported by a number of authors. Zaumeyer and Wade (18, 19) mechanically transmitted a virus causing mosaic in white clover to a number of hosts. Inoculation of bean (*Phaseolus vulgaris* L.) showed both necrotic local lesions on the primary leaves and a systemic mottling of trifoliate leaves. Pierce (10, 11) reported a similar reaction on bean when inoculated with a white clover virus which he designated as white clover virus 1. He did not obtain infection on *Nicotiana tabacum*. This virus was later named Trifolium virus 1 by Weiss (17). E. M. Johnson (5) worked with a white clover mosaic virus which produced only local lesions on bean and infected tobacco. However, he could not reinfect white clover. F. Johnson (6) showed that the white clover mosaic virus with which he was working was in reality 2 distinct viruses which he called pea wilt and pea mottle. These could be separated by means of dodder. One virus was limited in host range to the family *Leguminoseae*, whereas the other had a wider host range. Kreitlow and Price (7) reported a strain of alfalfa mosaic virus that caused mosaic of white clover which they designated as yellow-patch mosaic. The virus had a thermal inactivation point slightly higher than that found for similar viruses described by Valleau (16) and Johnson (5). The latter 4 authors reported tobacco as being susceptible. Oswald (9) reported a Ladino clover mosaic virus that caused a type of vine and tuber necrosis in potato which differed slightly from that caused by isolates of alfalfa mosaic virus from alfalfa yet showed similar reactions upon other hosts and had the same host range. He concluded that the Ladino clover mosaic virus was a strain of alfalfa mosaic virus. A number of authors have shown white clover to be susceptible to alfalfa mosaic virus (7, 9, 11).

VIRUS COLLECTIONS AND METHODS OF TRANSMISSION.—Collections of mosaic diseased Ladino clover and alfalfa were made from various locations in California. Collections of potato plants with calico and others with tuber necrosis were also obtained. The viruses from field collections were first transmitted to the Bountiful variety of bean (*Phaseolus vulgaris* L.) by juice inoculation. All bean plants tested were grown in sterile soil and inoculated by rubbing primary leaves with extracted juice and employing carborundum as in the method described by Rawlins and Tompkins (12). A preliminary report by the authors (4) indicated that the viruses commonly present in the above mentioned plant collections could readily be divided into 4 types on the basis of symptoms on Bountiful bean and Ladino clover, host range, thermal inactivation, and cross protection. Two of the viruses appeared to be alfalfa mosaic strains and 2 yellow-bean mosaic strains. On the basis of their reaction on Bountiful bean, these 4 strains will hereafter be referred to as: 1—local lesion alfalfa mosaic virus; 2—systemic alfalfa mosaic virus; 3—necrotic yellow bean mosaic virus; 4—mild yellow bean mosaic virus. In reality the 4 strains appear to be made up of isolates that show minor variations in symptom expression on Bountiful bean and Ladino clover. Isolates to be used in further studies were selected as representative of these strains.

Separation of the viruses from field samples.—Most field collections of Ladino clover contained more than 1 of these virus strains (Table 1) as did also some of the alfalfa and potato samples. Bountiful bean proved to be one of the best hosts for the separation of the virus strains because of its susceptibility and distinct

TABLE 1.—*Relative prevalence of 4 virus strains on the basis of symptom expression on Bountiful bean plants mechanically inoculated with juice extracted from field collections of virus diseased Ladino clover, alfalfa and potatoes*

Virus source from field	Symptom expression on beans and relative prevalence in field			
	Alfalfa mosaic virus		Yellow bean mosaic virus	
	Local lesion	Systemic	Necrotic	Mild
Ladino clover				
1	+	—	+	+
2	+	—	—	—
3	+	+	+	—
4	+	—	—	—
5	+	—	+	—
6	+	+	+	—
7	+	—	+	—
8	+	—	—	—
9	+	—	+	—
10	+	+	+	+
11	+	—	+	—
Alfalfa				
1	+	—	—	—
2	+	—	—	—
3	+	—	—	—
4	+	—	—	—
5	+	+	—	—
Potato				
1	+	—	—	—
2	+	—	—	—
3	+	+	—	—
4	+	+	—	—

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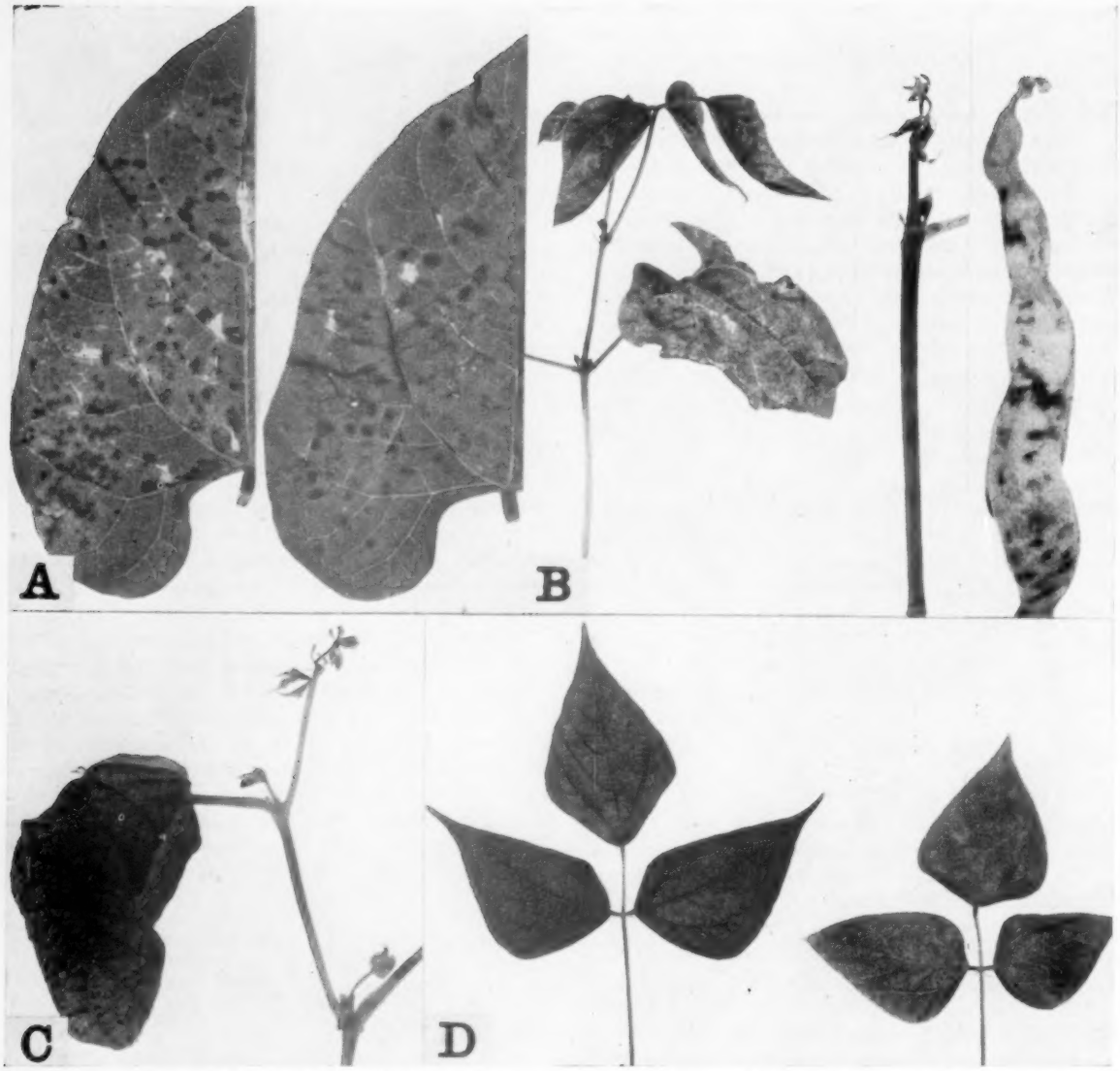

FIG. 1. Symptoms produced on Bountiful beans by 4 viruses isolated from Ladino clover. A) Two types of local lesions caused by local lesion strains of alfalfa mosaic virus; left, necrotic rings with tan centers; right, large diffuse spots and veinal necrosis. B) Systemic alfalfa mosaic virus; left, necrosis and reddening of primary node and systemic yellow mottle in first trifoliate leaf—note absence of local lesions on inoculated leaf; center, necrosis of growing point and stem; right, red pod mottle and distortion. C) Necrotic yellow bean mosaic virus showing downward rolling of trifoliate leaflets and complete necrosis of growing point. D) Mild yellow bean mosaic virus; left, healthy; right, diffuse netted chlorotic mottle.

symptom expression. The systemic alfalfa mosaic strains were readily separated from the local lesion strains by sub-inoculations from the systemic phase. The local lesion strain was obtained in a pure form from Ladino clover samples that previously had been found not to contain the systemic strain. The systemic alfalfa mosaic and yellow bean mosaic viruses could be separated by inoculation of young celery plants (var. Cornell Golden) with juice from systemically infected bean plants since celery is not a host of the yellow bean mosaic virus. The yellow bean mosaic virus strains were separated from each other by inoculation of several Bountiful bean plants which invariably resulted in some plants exhibiting symptoms of only the necrotic strain whereas other plants showed only the mild strain.

SYMPTOMS OF THE FOUR VIRUS STRAINS ON BOUNTIFUL BEAN.—*Local lesion alfalfa mosaic virus strains.*—The results in Table 1 show that all 20 collections of Ladino clover, alfalfa and potato contained a virus which caused local lesions when juice inoculations were made onto primary leaves of Bountiful bean (Fig. 1A). The local lesions appeared in about 48 hours and developed into 2 rather distinct types, similar to those described by Oswald (9). One type was nearly circular in outline with a dark-brown necrotic ring 1–2 mm.

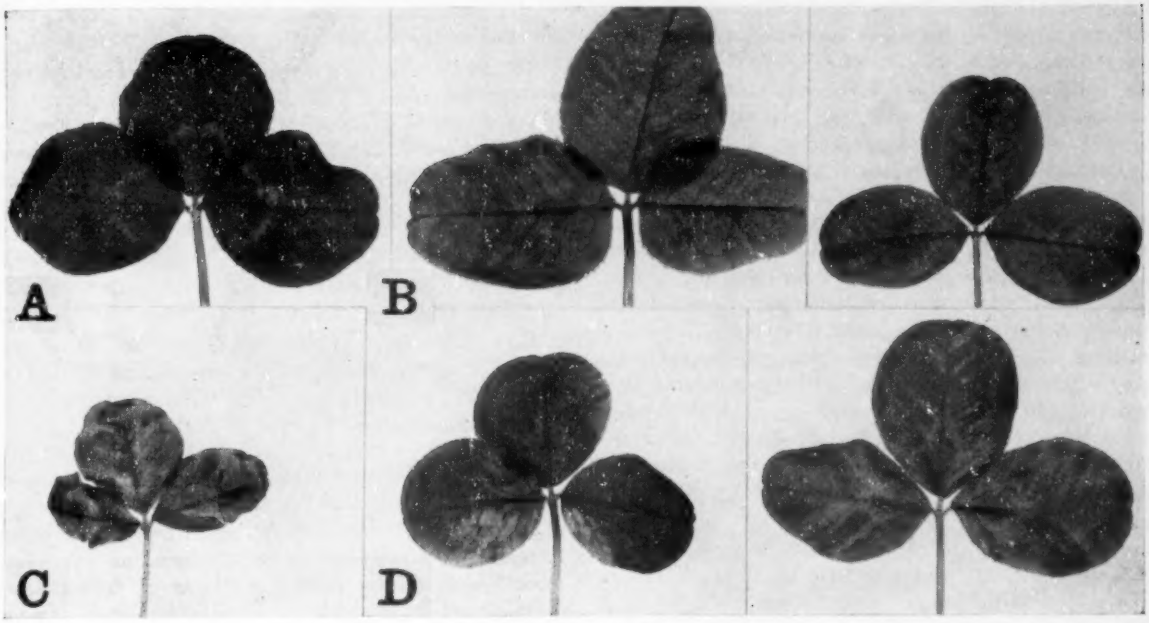

FIG. 2. Ladino clover leaves showing symptoms caused by 5 virus strains isolated from naturally infected Ladino clover. A) Healthy leaf. B) Left, necrotic, yellow bean mosaic virus; right, mild yellow bean mosaic virus showing chlorotic flecks between the veins. C) Local lesion strain of alfalfa mosaic virus with severe distortion and mottle. D) Two strains of systemic alfalfa mosaic virus; left, yellow blotches starting at leaf margin and proceeding inward between veins; right, similar blotches, but not delimited as much by veins.

in diameter with green centers later becoming tan. Some of the smaller lesions failed to show distinct centers. The other type consisted of lesions 4–5 mm. across, very irregular in outline, netted and reddish-brown in color and often showed, on the lower surface of the leaves, veinal necrosis extending beyond the margins of the necrotic spot. Juice from single original plant samples usually produced local lesions of only 1 type; however, a few samples produced local lesions of both types.

Systemic alfalfa mosaic virus strains.—These virus strains were recovered from all 3 hosts collected in the field and in 6 of the 20 collections (Table 1). No necrotic local lesions were produced on Bountiful bean by these strains. The first symptoms in bean appeared 7–10 days after inoculation and were exhibited as a reddish-brown internal necrosis of the petioles of the inoculated leaves with the necrosis pronounced at the nodes and extending in both directions in the main stem of the plant. Necrosis next developed in the petiolules and veins of the young trifoliate leaves which soon abscissed. In some cases complete collapse and death of the growing point occurred (Fig. 1-B). Chlorotic spots often developed in the leaflets preceding necrosis of the veins in that area. Plants recovering from the original necrosis were extremely stunted with irregular chlorotic blotches in the expanding leaves and if pods developed, they were often malformed and showed reddish-brown irregular blotches on the surface (Fig. 1,B). With some isolates, inoculated leaves exhibited faint chlorotic rings or partial rings 1–3 mm. in diameter sometimes with pin-point necrotic centers.

Necrotic yellow-bean mosaic virus strains.—These virus strains were isolated from 8 of 11 Ladino clover samples tested, but were not obtained from the samples of alfalfa or potatoes (Table 1). No local lesions developed on Bountiful bean. The first symptoms on bean appeared in 7–10 days at the growing point with the expanding leaflets dark in color and curled downward at the margins. This was followed by necrosis of the leaflets, petioles, and growing point with death of the plant resulting in most cases (Fig. 1,C). Those plants which survived, produced small distorted leaflets with extreme chlorotic mottle. Pods on such plants were malformed and markedly roughened. In later stages of the disease inoculated leaves often showed vein chlorosis with adjacent necrotic bands.

Mild yellow bean mosaic virus strains.—These virus strains were obtained from only 2 of the Ladino clover collections and not from alfalfa or potato (Table 1). Systemic symptoms developed on Bountiful bean 6–8 days after inoculation of the primary leaves. The symptoms consisted of small chlorotic areas irregular in shape and with chlorosis most pronounced adjacent to the veins, thus resulting in a netted appearance (Fig. 1,D).

SYMPTOMS AND EFFECT ON YIELD OF THE VIRUS STRAINS ON LADINO CLOVER.—When representative isolates of the 4 virus strains were separately inoculated into Ladino clover it was found that each strain produced rather distinct symptoms except for the 2 yellow bean mosaic strains which reacted quite similarly (Fig. 2,B). The local lesion strain of alfalfa mosaic

virus produced the most pronounced effect with marked stunting, large irregular chlorotic blotches and a striking rugosity of the leaves (Fig. 2, C). The systemic alfalfa mosaic strains caused only a slight stunting of the plant with the first symptoms developing on the new leaves as gray tissues between the lateral veins of the leaflet. Later the leaves showed bright yellow blotches starting at the leaflet margin and extending toward the midrib. The leaflet veins remained green in early stages but later most of the tissues in the chlorotic area lost their chlorophyll (Fig. 2,D). The yellow bean mosaic strains caused less stunting than the alfalfa mosaic strains. Symptoms consisted of chlorotic flecks between the lateral veins and a slight puckering of the interveinal tissues, thus rendering the main veins more prominent both in color and position (Fig. 2,B). These 4 viruses used in the original inoculation were readily recovered from the Ladino clover plants when inoculated back to Bountiful bean.

Green plant yields taken from inoculated blocks of 45 Ladino clover plants grown in the greenhouse showed that of the single virus strains tested the local lesion alfalfa mosaic strains caused the most severe stunting with yields about ⅔ that of the checks. The systemic alfalfa and yellow bean mosaic strains reduced yields very slightly. Blocks inoculated with juice from Ladino clover carrying a mixture of the local lesion alfalfa mosaic virus and the 2 yellow bean mosaic viruses caused the most severe stunting with yields less than ½ that of the checks.

SYMPTOMS OF THE VIRUS STRAINS ON BEAN, BROAD BEAN AND ALFALFA.—Results of inoculation of 9 bean varieties (*Phaseolus vulgaris* L), broad bean (*Vicia faba* L) and alfalfa (*Medicago sativa* L) aided in distinguishing the 4 virus strains.

Local lesion alfalfa mosaic virus.—This strain produced only local lesions, similar to those described on Bountiful bean, on all 9 bean varieties. On broad bean, local lesions were also produced as well as a systemic chlorotic mottle followed by reddish necrotic streaks on the stems near the growing point. Alfalfa proved difficult to inoculate, as other investigators (5, 7, 9) have found. The percentage of infected alfalfa seedlings varied from 0 to 50 and those affected exhibited chlorotic rings with lateral chlorotic extensions into the leaf tissues. The virus was recovered from such plants by transmission to Bountiful bean.

Systemic alfalfa mosaic virus.—Local lesions were produced on all the bean varieties tested with the exception of Bountiful. The lesions were large, 4–6 mm. in diameter with a netted appearance resulting from a dark brown network of veinal necrosis. Similar lesions sometimes appeared on trifoliate leaves as part of the systemic phase. The systemic necrosis and chlorotic mottle were similar on all varieties and much as described on Bountiful bean. Local lesions were produced on broad bean followed by a yellow mottle and a reddish necrosis of the young tissues and usually subsequent death of the plants. The few plants of alfalfa that became infected showed yellow streaks and blotches on the leaves.

Necrotic yellow bean mosaic virus.—No local lesions were produced on any of the bean varieties. Systemic symptoms on all susceptible varieties were very similar to those described on Bountiful bean. Great Northern U.I. 15, U.I. Pinto 11, Small Flat White, and Bountiful were susceptible, whereas the varieties, G.N. U.I. 16, 31, 59, 81 and 123 were resistant. Broad bean was susceptible and developed extremely rugose leaves with a bright-yellow chlorotic mottle. No infection was obtained on alfalfa.

Mild yellow bean mosaic virus.—Infected beans exhibited a mild chlorotic mottle accentuated along the veins as on Bountiful bean. The relative susceptibility of bean varieties was exactly as that described above for the necrotic strain. A very mild mottle was produced on broad bean and no infection was obtained in alfalfa.

SYMPTOMS OF VIRUS STRAINS ON HOSTS OTHER THAN LEGUMES.—No symptoms were produced on any hosts other than legumes by the 2 strains of yellow bean mosaic virus. The alfalfa mosaic strains produced symptoms on celery (*Apium graveolens* L. var. Cornell Golden), pepper (*Capsicum frutescens* L. var. California Wonder Bell), potato (*Solanum tuberosum* L. var. White Rose), tobacco (*Nicotiana tabacum* L. var. Turkish) and *N. glutinosa* L. On these hosts the isolates of both alfalfa strains resulted in symptoms similar to those described by other investigators (2, 5, 9, 13, 16). In no case were local lesions produced on tobacco or *N. glutinosa*. On all of these hosts the 2 types of alfalfa virus were indistinguishable. On potato, isolates of both strains resulted in symptoms varying from the true calico to tuber and vine necrosis (9).

CROSS-PROTECTION TESTS.—Complete cross-protection was obtained on Bountiful bean when the local lesion strain of alfalfa mosaic virus was inoculated onto plants which had 10 days previously been inoculated with a systemic strain. The mild and necrotic yellow bean mosaic viruses failed to protect against any of the alfalfa mosaic strains. This failure of cross-protection was also true in the reverse order. The mild strain of yellow bean mosaic virus failed to protect against the necrotic strain in several repeated trials. It was impossible to try cross-protection in the reverse order because the severity of the necrotic strain resulted in death of the bean plants or obscurity of the mild reaction, if such occurred. It was not possible to use hosts other than bean in cross-protection tests because of the difficulty to distinguish between symptoms of the 2 strains on such hosts.

PROPERTIES OF THE VIRUS STRAINS.—*Thermal inactivation.*—The temperatures required to inactivate the virus strains when heated for 10 minutes in a water bath were determined. Juice from infected Ladino clover was used for the local lesion alfalfa mosaic strain and from infected Bountiful bean for the other strains. Bountiful bean plants were inoculated with the treated samples to determine the temperature of

inactivation. Check plants inoculated with aliquots of the unheated samples before and after the heating trials were all infected. The local lesion strain of alfalfa mosaic virus originating from Ladino clover showed inactivation between 55 and 58°C. This was identical to a strain recovered from white clover by Johnson (5), and by Oswald (9), and similar to alfalfa mosaic N as reported by McWhorter (8). The 2 systemic strains of alfalfa mosaic virus tested were inactivated at 61–64°C. which is identical to that reported by Pierce (10), Zaumeyer and Wade (19), Kreitlow and Price (7) and Berkeley (1) for alfalfa mosaic strains which they studied. Both strains of yellow bean mosaic were inactivated at 58–61°C. This is the same as that reported for bean virus 2 by Pierce (10) and for the pod-distorting strain of bean virus 2 described by Grogan and Walker (3).

Longevity in vitro of alfalfa mosaic strains.—Bountiful beans were used as the test host to determine when virus activity was lost in juice samples stored at 16°C. Inoculations were made with this juice at 24 hour intervals. Most strains of alfalfa mosaic virus lost their infective quality between 72 and 96 hours. One local lesion strain lost its infectivity between 48 and 72 hours.

IDENTITY OF THE VIRUSES.—*Local lesion alfalfa mosaic virus.*—In attempting to classify the viruses herein described, it became evident that there was present in the literature considerable confusion as to possible synonymy of the viruses affecting legumes. It was very difficult to determine the relationship of one virus to another with any degree of exactness. However, it appears that the virus strains under discussion which produced only local lesions on bean were strains of alfalfa mosaic. This conclusion was based on thermal inactivation, host range, and symptom expression. The fact that virus strains of this type obtained from naturally infected alfalfa exhibiting the mosaic disease appeared identical to those obtained from Ladino clover mosaic and from potato calico and tuber necrosis further substantiates their classification as alfalfa mosaic strains. Furthermore, strains of alfalfa mosaic virus from alfalfa protected against infection by those from Ladino clover. Symptom expression on the various hosts inoculated indicate that these strains were similar to the alfalfa mosaic virus affecting celery as reported by Snyder and Rich (13) and those strains producing calico and tuber necrosis of potato reported by Oswald (9) and calico of potato described by Black and Price (2). The local lesion effect on bean was the same as that reported by a number of authors (4, 5, 6, 9, 10). On Ladino clover the local lesion strains resulted in production of symptoms similar to those described by Johnson (5) and Valleau (16).

Systemic alfalfa mosaic virus.—The alfalfa mosaic strains systemic on bean had thermal inactivation points between 61 and 64°C. This is the same as that shown by Kreitlow and Price (7), Pierce (10) and Zaumeyer and Wade (19) for the alfalfa mosaic strains with which they were working. The systemic strains protected bean plants from infection by the local lesion strains. Symptom expression by the local lesion and systemic strains was found to be identical on celery, tobacco, and potato, but differed on bean and Ladino white clover. The systemic strains appeared to be similar to that described by McWhorter (8) as alfalfa N virus except that they showed a slightly higher thermal inactivation temperature than did his strain. Symptom expression on bean was about the same as that described by Thomas and Zaumeyer (15) for red node, but the alfalfa virus differed in having a higher thermal death point and in being infectious to *N. glutinosa*, celery, and white clover. The systemic alfalfa mosaic strains described in this paper also seem to be very closely related to the alfalfa mosaic strain described by Thomas (14) as yellow dot virus on bean, although the 2 differed somewhat in their reaction on certain bean varieties.

Necrotic yellow bean mosaic virus.—The necrotic yellow bean mosaic strains obtained from Ladino clover appeared to be very nearly the same as the pod-distorting strain of yellow bean mosaic virus reported by Grogan and Walker (3). They were identical in symptoms, host range, relative susceptibility of bean varieties tested, and thermal inactivation points. The only difference shown was the fact that the pod-distorting virus failed to infect broad bean.

Mild yellow bean mosaic virus.—The mild strain of yellow bean mosaic virus appeared to be the same as typical bean virus 2 (3, 10) and the necrotic yellow bean mosaic virus in respect to thermal inactivation, host range, and relative susceptibility of bean varieties tested. It differed from these in that the symptom expression on bean was very mild. It appeared similar to Johnson's pea-wilt virus obtained from white clover (6) and the white clover mosaic virus of Kreitlow and Price (7).

DISCUSSION.—From these investigations, it is evident that the Ladino variety of white clover exhibiting mosaic symptoms may be naturally infected by a number of viruses and virus strains and that single Ladino clover plants may contain several of the viruses at the same time. There is good evidence in the literature, as well as that described in this paper, that alfalfa mosaic virus may consist of many strains. The local lesion and systemic strains, herein discussed, appear to be in reality a number of different strains which have been placed in 2 groups on the basis of their symptom expression on bean and Ladino clover. Inoculation of White Rose potatoes by single isolates of each group of strains have shown that some produce typical calico symptoms, others calico plus varying degrees of stem and leaf necrosis, while still others produce typical stem and tuber necrosis with no evidence of calico. The 2 groups were indistinguishable on potato; however, they could be separated upon the basis of their reactions on bean and white clover. Considerable difference in thermal inactivation temperatures of alfalfa mosaic strains has been shown by a number of authors (1, 2, 7, 8, 9, 11) as well as in this paper. The fact

that such marked differences can occur in strains comprising a single virus further helps to explain differences which have been found in regard to host range and symptom expression when certain hosts are tested. In fact such differences must be expected when working with a virus of this type.

It appears that there is no true white clover mosaic virus, as described by Pierce (10, 11), but that the viruses present in Ladino white clover in California are strains of viruses previously described on other hosts. Strains of alfalfa mosaic and yellow bean mosaic viruses are the important ones present in the Ladino-clover complex.

SUMMARY

The Ladino variety of white clover shows a number of different mosaic symptoms in the field in California. At least 4 virus strains have been isolated from such plants by juice inoculation onto Bountiful bean. Eight of 11 samples selected from various fields on the basis of symptom expression contained more than 1 strain of virus. One virus produced only local lesions on all varieties of beans tested; another produced no local lesions on Bountiful bean, but did so on all other varieties used and also produced a systemic necrosis and mottle on all the varieties. Different symptoms were produced on Ladino clover by these 2 strains but they were indistinguishable from each other on alfalfa, broad bean, celery, potato, and tobacco. The 2 strains had slightly different thermal inactivation points but cross protected against each other. Cross protection was also obtained between these isolates and isolates from alfalfa showing typical mosaic symptoms. On the basis of symptoms on several hosts, host range, thermal inactivation, and cross protection, they may be classified as 2 strains of alfalfa mosaic virus. The 2 alfalfa mosaic strains appeared to be composites of many strains on the basis of their reaction on potato. These same 2 virus strains were obtained from 5 naturally infected alfalfa plants showing mosaic. All 5 samples tested carried the local lesion alfalfa mosaic virus strain as expressed on Bountiful bean and 1 sample also contained the systemic alfalfa mosaic virus strain. Four samples of potato, some showing calico and others tuber necrosis, were found to carry the local lesion virus strain and 2 of the samples also contained the systemic virus.

Two other virus strains isolated from Ladino clover were found not to infect hosts tested other than those in Leguminoseae. One strain caused severe necrosis and yellow mottle of beans, whereas the other strain caused only a mild yellow mottle. On Ladino clover these strains produced a slight stunting with leaves showing small interveinal, chlorotic spots and a slight roughening. Such isolates were the same as bean virus 2 in regard to host range, susceptibility of bean varieties, and thermal inactivation points and were thus considered strains of yellow bean mosaic virus.

All the viruses obtained from Ladino clover seem to be related to already described viruses—either alfalfa mosaic virus or yellow bean mosaic virus.

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FUSARIUM WILT OF RADISH IN WISCONSIN

Glenn S. Pound and Dorsey L. Fowler [1]

In the summer of 1946, certain plantings of radish (*Raphanus sativus* L.) in Waukesha County, Wisconsin, were severely affected with a vascular wilt disease which had been evident for a few years prior to this but caused little concern. Isolations from diseased seedlings consistently yielded a fungus of the genus *Fusarium*. Greenhouse pathogenicity tests with single spore cultures of these isolates produced symptoms identical to those found in nature.

A *Fusarium* wilt of radish has also been reported from California by Kendrick and Snyder (3) and later by Snyder and Barden (7). They named the radish pathogen *Fusarium oxysporum* f. *raphani* n. f. A culture of this organism was obtained from Dr. Snyder and parallel studies of it and the Wisconsin radish isolate indicated that they were identical. Hence the incitant of the disease in Wisconsin is considered to be the *F. oxysporum* f. *raphani* of Kendrick and Snyder. A preliminary report of the disease in Wisconsin has already been made (4).

SYMPTOMS AND HOST RANGE.—The first evidence of the disease in the field is found on very young seedlings. It is characterized by a rapid yellowing and wilting of the cotyledons and dying of the roots. The stem at first may remain turgid and, when the entire plant is dead, the stiffened stem often stands erect with the leaves attached but drooping. If conditions are not favorable for attack in the seedling stage, older leaves are yellowed, especially along the veins, and often show a unilateral development. Cross sections or longitudinal sections of the root, stem, hypocotyl, and leaf petiole reveal a discoloration of the vascular ring or of single vascular strands (Fig. 1, C). Root enlargement may be completely suppressed and may never reach more than pencil size (Fig. 1, A).

Pathogenicity tests with the Wisconsin radish pathogen in comparison with the cabbage yellows pathogen (*Fusarium oxysporum* f. *conglutinans* Wr.) Sny. and Han. revealed that the 2 fungi produced identical diseases on radish, regardless of the method of inoculation. The 2 fungi were tested in parallel inoculations to a number of cruciferous hosts, the results of which are in Table 1. In greenhouse tests roots of seedling plants grown in sand were washed and dipped in a mycelial-spore suspension after which the plants were transplanted to clean sand and incubated at a soil temperature of 22° C. Nutrient solutions were applied to the sand. In field tests, the same seed lots were sown respectively on soil infested with the cabbage and radish strains of the fungus.

It can be seen from Table 1 that both isolates are infectious to a wide range of cruciferous plants. Greenhouse tests were made with seedling plants and under severe artificial inoculation. Since these results might not reflect the reaction the species would give in the field, plantings were made on soil naturally infested with the respective pathogens. Very similar results were obtained but the percentage of infection and disease development were generally lower than in greenhouse seedling tests. Much of the infection was detectable only by transecting the roots but with the cabbage yellows organism occasional plants of all susceptible species developed very conspicuous "yellows" symptoms. This was also the case with the radish organism except that no macroscopic symptoms were evident on any member of the cabbage tribe. Isolations from affected plants in field tests yielded the respective strains.

The cabbage yellows organism has generally been considered to be restricted to members of *Brassica oleracea*. It is evident that it is widely infectious within the cruciferae but that on some of these hosts it is much less pathogenic than on cabbage. On radish, however, it is very severe.

The radish strain is much less pathogenic than the cabbage strain, except on radish on which it is slightly more severe. It was practically noninfectious to members of the cabbage tribe except to kohlrabi in the greenhouse tests. Symptoms of the 2 organisms on the various hosts were identical. As shown in the following section, the radish organism will infect cabbage at high soil temperatures. Thus, if greenhouse tests had been made at 28° rather than 22°, many members of *B. oleracea* might have been susceptible.

EFFECT OF AIR AND SOIL TEMPERATURE.—Single spore cultures of the 2 fungi were grown on potato-dextrose agar plates at air temperatures of 8°, 12°, 16°, 20°, 24°, 32°, 36°C. In each of 3 trials it was found that the optimum temperature for each isolate was 24°–28°C. The 2 fungi were easily separable on P.D.A. by the pink pigmentation produced by the radish strain.

In soil temperature tests, the 2 organisms were tested against Copenhagen Market cabbage (yellows susceptible), Improved Wisconsin Ballhead cabbage (yellows resistant), Scarlet Globe radish, and a line (SG 121A) of Scarlet Globe radish selected as resistant to the radish organism. Four trials were made; representative data are in Table 2.

In each trial it was found that certain distinct differences exisited in the pathogenicity of the 2 organisms. The cabbage yellows organism on susceptible cabbage and on the 2 lots of radish showed a gradient of severity that increased as soil temperature increased. Although the ultimate severity of symptoms was about the same at 24°, 28°, and 32°, the rate of disease development increased with increase in temperature. The

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TABLE 1.—*Host range and pathogenicity of the cabbage and radish wilt Fusaria*

Host	Greenhouse Tests [a]				Field Tests [b]	
	Percentage of plants infected [c]		Disease index [d]		Percentage of plants infected [c]	
	Cabbage isolate	Radish isolate	Cabbage isolate	Radish isolate	Cabbage isolate	Radish isolate
Cabbage (Copenhagen Market)	100	0	100	0	70	0
Cabbage (Wisconsin Copenhagen)	0	0	0	0	0	0
Kale (Dwarf Scotch Curled)	100	0	100	0	44	3
Brussels Sprouts (Long Island Improved)	—	—	—	—	33	0
Cauliflower (Early Snowball)	100	0	97	0	29	0
Broccoli (Italian Green Sprouting)	81	7	58	2	24	0
Kohlrabi (White Vienna)	100	45	82	11	25	5
Rutabaga (American Purple Top)	94	72	59	49	29	4
Turnip (Purple Top White Globe)	93	60	70	40	32	9
Mustard (Giant Southern Curled)	79	100	53	100	30	36
Rape (Dwarf Essex)	73	53	27	12	4	2
Chinese Cabbage (Michili)	89	84	35	63	28	3
Garden Cress	—	—	—	—	39	16
Stocks (Annual 10 weeks)	67	100	47	93	—	—
Radish (Early Scarlet Globe)	87	93	58	93	58	52

[a] = Notes taken 30 days after inoculation; soil temperature 22° C.
[b] = Notes taken 30 days after seeding; values are averages of 2 trials for the cabbage isolate and one trial for the radish isolate.
[c] = Determined by root discoloration when transected.
[d] = 0= No infection; 100 = death of all plants. Values are rounded off to the nearest decimal.

line of Scarlet Globe radish selected as resistant to the radish organism was also quite resistant to the cabbage organism. The radish organism was infectious to cabbage only at high soil temperatures. Slight stunting of infected plants occurred but, generally, infection was evident only as a slightly discolored vascular tissue of the root and lower stem. Isolations from these discolored areas readily yielded the fungus.

On Scarlet Globe radish, the radish organism was ultimately about as severe at one temperature as another. Disease development was most rapid at high temperatures but at 16° and 20° it developed much faster and was much more severe than that produced by the cabbage organism. The 2 pathogens were markedly distinct in this regard. In each of the 4 trials, the disease on radish incited by the radish organism at 32° and 28° was less than that at 24° which appeared to be the optimum soil temperature for disease development.

RADISH VARIETAL REACTIONS.—Fifteen commercial varieties of radish and many foreign introductions were tested against the radish wilt incitant in field plantings on infested soil and in greenhouse plantings with artificial inoculations. The following varieties were tested and found to be susceptible: Early Scarlet Globe, Chinese White Winter, Chinese Rose Winter, Sparkler, Icicle, French Breakfast, Comet, Cavalier, Round Black Spanish, Crimson Giant, Cincinnati Market, Scarlet Turnip White Tipped, White Strasburg, Long Scarlet, and Cherry Belle. Little difference existed

TABLE 2.—*Relation of soil temperature to disease development in cabbage and radish inoculated with the cabbage and radish wilt Fusaria*

Pathogen and Host	Disease index [a] at soil temperature					Average index
	16°	20°	24°	28°	32°	
Cabbage isolate						
Copenhagen Market Cabbage	18	40	99	100	98	71.0
Improved Wisconsin Ballhead Cabbage [b]	0	0	8	88	100	39.2
Scarlet Globe Radish	17	18	84	91	96	61.2
SG-121A Radish [c]	4	8	33	46	52	28.6
Average index	10	17	56	81	87	
Radish isolate						
Copenhagen Market Cabbage	0	0	0	10	19	5.8
Improved Wisconsin Ballhead Cabbage [b]	0	0	0	3	4	1.4
Scarlet Globe Radish	69	74	87	84	78	78.4
SG-121A Radish [c]	10	8	14	20	27	15.8
Average index	20	21	25	29	32	

[a] So calculated that if all plants showed none, slight, moderate, severe, lethal symptoms the index would be 0, 25, 50, 75, 100, respectively. Figures are rounded off to the nearest decimal. Notes were taken 23 days after inoculation.
[b] Yellows-resistant.
[c] Mass selected for resistance to the radish-wilt organism.

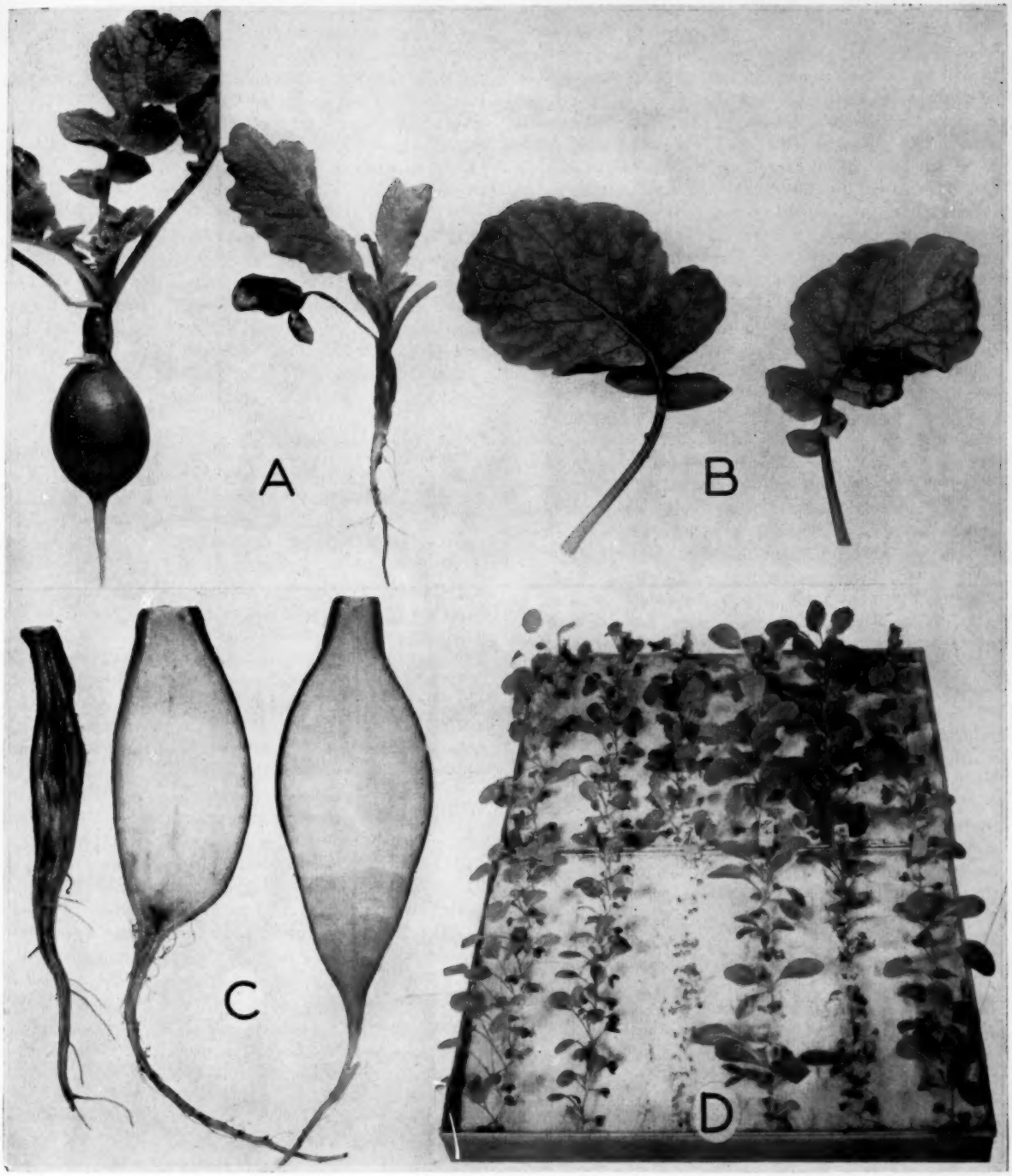

FIG. 1. A) Healthy plant of Scarlet Globe radish (left) compared with one naturally infected with the radish organism (right); B) radish leaves from plants infected with the radish organism (left) and the cabbage organism (right); C longitudinal sections of white Icicle radish infected with the radish organism (left and center) compared with healthy plant (right); D) greenhouse host range test with the radish organism (below) compared with healthy plants (above). Hosts are, left to right, broccoli, cauliflower, mustard, Chinese cabbage, radish, and cabbage.

among varieties in the percentage of plants susceptible. All varieties appeared to be heterozygous and to have occasional plants which remained free from infection.

Of the foreign introductions tested, none was immune, but many possessed a relatively high level of resistance. The most resistant ones were P.I. 161148, 159382, 159384, 161146, and 161379. These were all of very undesirable horticultural type, having long tops,

long roots, and being of late maturity.

CONTROL.—Since radish requires only 3–4 weeks from seeding to harvest, it was thought that perhaps by chemical seed treatment the soil surrounding the seedling roots could be kept disinfested long enough to allow the plants to remain healthy until harvest. A randomized and replicated field planting was made in which 50 ft. replicate rows were sown with seed treated with various fungicides and with seed to which formaldehyde was added in the row. The mean percentage infection of emerged seedlings for the various treatments was as follows: Cuprocide 30.7, New Improved Ceresan 32.8, Semesan Jr. 35.5, Arasan 40.8, formaldehyde drip 40.9, Phygon 44.8, untreated 45.5. Cuprocide caused distinct stunting of seedlings. Although the percentage of diseased plants was significantly reduced by some of the treatments, the reduction was not considered sufficient for practical control.

In 1947 a portion of a severely-affected field of Early Scarlet Globe was rogued and allowed to go to seed. Seed saved from several individual plants when tested in the greenhouse showed a marked increase in resistance. Certain inbred lines from these selections show complete resistance in greenhouse and field tests. Because of extreme difficulties encountered in getting seed sets from self pollinations, sib matings have for the most part been used. Several resistant lines of Scarlet Globe type have been thus derived and are being further tested and selected for resistance and type. It is believed that the disease can be readily controlled by resistant varieties and that resistance can be established in most if not all varietal types by selection.

DISCUSSION.—Kendrick and Snyder (3) named the incitant of radish wilt *Fusarium oxysporum* f. *raphani* n. f. because of its high pathogenicity to radish and its nonpathogenicity to cabbage and kale, thus distinguishing it from *Fusarium oxysporum* f. *conglutinans* (Wr.) Sny. and Han. The latter organism, although capable of attacking radish, showed highly selective specificity to members of *Brassica oleracea*. Baker (2) also named a *Fusarium* inciting wilt of garden stock (*Mathiola incana*) as *Fusarium oxysporum* f. *mathioli* n. f. because of its selective pathogenicity to stock and its nonpathogenicity to cabbage. Snyder (5) made cross inoculation studies with the 3 organisms and found that occasional cross infection occurred but that each showed a highly selective specificity for its own host. Therefore, he considered the 3 organisms to be forms of *Fusarium oxysporum* Schl. emend. Sny. and Han.

Armstrong and Armstrong (1) on the basis of cross inoculation studies with the cabbage and radish fusaria showed that cabbage, radish, and stock could be infected with each organism. They listed the cabbage, radish, and stock fusaria as races 1, 2, and 3, respectively, of *Fusarium conglutinans* Wr.

In view of the fact that both cabbage and radish isolates are widely infectious among the Cruciferae, as shown here, and that distinct differences in pathogenicity occur, the writers prefer to list them as races 1 and 2, respectively, of *Fusarium oxysporum* f. *conglutinans* (Wr.) Sny. and Han. Since the stock isolate also can infect hosts common to the cabbage and radish isolate (5) it should be listed as race 3 of the species *F. oxysporum* f. *conglutinans*.

SUMMARY

A destructive radish wilt in Wisconsin is caused by a vascular *Fusarium*. The disease is manifested by chlorosis, necrosis, and abscission of leaves and by vascular discoloration of roots, stems, and petioles. Root enlargement is markedly suppressed.

In comparing the radish pathogen with the incitant of cabbage yellows, it was found that both organisms attacked radish, stock, turnip, rutabaga, rape, Chinese cabbage, cress, mustard, and kohlrabi. The radish organism did not attack cauliflower or kale and only occasional plants of broccoli. These last named hosts were all susceptible to the cabbage organism. Cabbage was not attacked by the radish organism except at soil temperatures of 28° and 32°.

In comparative soil temperature studies it was found that the radish organism was much more active at lower temperatures than the cabbage organism.

The cabbage and radish wilt fusaria are listed as races 1 and 2, respectively, of *Fusarium oxysporum* f. *conglutinans* (Wr.) Sny. and Han.

All commercial varieties of radish tested were found susceptible to the radish organism with little difference existing between varieties.

Resistant lines of Scarlet Globe type have been developed by selection.

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ROLE OF CHELATION IN CAUSING AND INHIBITING THE TOXICITY OF LYCOMARASMIN [1]

Paul E. Waggoner and A. E. Dimond

Lycomarasmin, produced in culture filtrates of *Fusarium oxysporum* f. *lycopersici*, is toxic to tomato cuttings and has been suggested as a vivotoxin in Fusarium wilt of tomatoes (5). Initially isolated and partially characterized by Plattner and Clauson-Kaas (7), lycomarasmin has been shown (9) to be dipeptide of asparagine and a new amino acid, N-(α-(α-glycyl) hydroxypropionic acid. The peptide bond occurs between the amino group of asparagine and the carboxyl group of the glycine moiety.

Lycomarasmin has a distinctive characteristic: it is 10 times as toxic as tomato cuttings if applied together with iron rather than alone (5). No matter what the manner of this "potentiation" a class of inhibitors of lycomarasmin can be predicted. Some chelating agents[2] have the ability to bind iron by forming complexes with relatively high stability constants. Consequently, if such a chelating agent were introduced into a system with lycomarasmin and a limited amount of iron, lycomarasmin toxicity should be decreased because less iron is available for potentiation.

If lycomarasmin is itself a chelating agent which is toxic only when the chelate complex with iron is formed, a second class of inhibitors can be predicted. It has been found that many chelating agents combine with metals in a definite order (6). Iron complexes are generally less stable than copper complexes. If lycomarasmin is a chelating agent of this common type, the quantity of iron-lycomarasmin should be small and the quantity of copper-lycomarasmin should be large in a mixture of copper and iron ions and a limited amount of lycomarasmin. Copper is known not to potentiate lycomarasmin (5). Therefore, if lycomarasmin is toxic only as the iron complex, lycomarasmin toxicity should be decreased when copper ions are introduced into a system with iron and a limited amount of lycomarasmin.

In the following studies we shall test the ability of the 2 classes of inhibitors to decrease lycomarasmin toxicity. First, 8-quinolinol, an agent which chelates both ferric and ferrous ions, will be tested as an inhibitor of the toxicity of lycomarasmin to tomato cuttings when the amount of iron is limiting. Then, after a demonstration that lycomarasmin forms chelate complexes with iron and copper, copper ion will be tested as an inhibitor of the toxicity of lycomarasmin to tomato cuttings when the amount of lycomarasmin is limiting.

Finally, the compounds which inhibit lycomarasmin when either the toxin or iron are limiting, are used in an attempt to decrease the severity of symptoms of Fusarium wilt. The inhibitors are expected to control any symptoms due to lycomarasmin because the activity of other metal dependent systems can be altered *in vivo* by changing the amount of metal ion available to the plant (2).

MATERIALS AND METHODS.—A sample of pure lycomarasmin (m.p. 228° C.) was kindly furnished by Dr. E. Gaumann. Reagent grade sulfate salts of iron and copper and Eastman grade 8-quinolinol were used.

Bonny Best tomato plants were used throughout these studies for cuttings or to provide diseased plants. In the latter case they were inoculated with a shake culture of *Fusarium oxysporum* f. *lycopersici* (3).

Hydrogen ion concentration was measured electrometrically and adjusted by adding sodium hydroxide. Solutions containing iron and lycomarasmin were adjusted to pH 6 before solutions of cupric sulfate or 8-quinolinol, also at pH 6, were added.

RESULTS AND CONCLUSIONS.—*Lycomarasmin inhibition by an iron chelating agent.*—To test whether a synthetic chelating agent will inhibit lycomarasmin when iron is limiting, 8-quinolinol was used. Preliminary experiments indicated that lycomarasmin alone was only slightly toxic (see also (5)), that lycomarasmin molecules plus ferrous ions in a ratio of 1 to 1 was more toxic, and that when the ratio was increased to 2 lycomarasmin molecules to 1 ferrous ion, toxicity was even greater. Accordingly, for systems in which iron was to be limiting, twice as many molecules of lycomarasmin as ferrous ions were used. It seemed reasonable to assume that if ferrous ion was limiting at a ratio of 2 to 1, ferric ion would be limiting at 3 to 1. Therefore, mixtures of lycomarasmin and iron ions were prepared in which the final concentrations were 9.25×10^{-4} M lycomarasmin and 4.62×10^{-4}N ferrous ion or 3.08×10^{-4} N ferric ion. One hour after the lycomarasmin and iron were mixed, 8-quinolinol was added to ½ of the mixtures to give a final concentration of 9.25×10^{-4} M. The dark iron-quinolinol was evident in all mixtures containing iron whether lycomarasmin was present or not. Two cuttings were then placed in each mixture.

After 20 hours all leaves of all plants placed in lycomarasmin and ferric or ferrous ion were severely and equally injured (Figure 1). In contrast, plants placed in iron and lycomarasmin to which 8-quinolinol had been added were identical with the checks (Figure 1). The cuttings in each mixture had taken up 5–8 ml. Uptake was not correlated with injury. The cuttings placed in solutions of iron salts, 8-quinolinol, or mixtures of iron and 8-quinolinol showed no injury and were identical in appearance with cuttings in water. The same was true of cuttings exposed to lycomarasmin alone or lycomarasmin plus 8-quinolinol.

Sixty-six hours after the cuttings were placed in the solutions, no injury was evident on any cuttings which

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[2] Albert (1) has reviewed the application of chelating agents in selective toxicity.

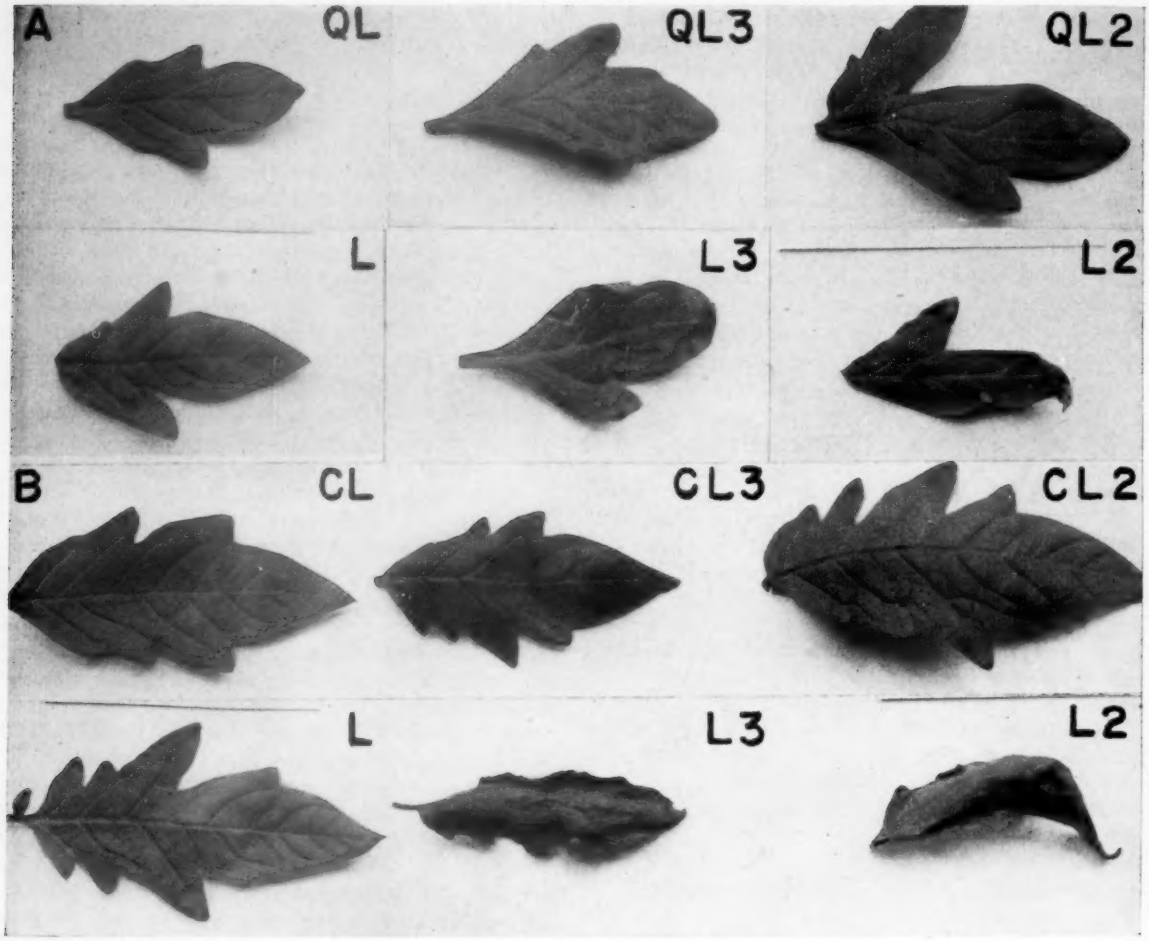

FIG. 1. Leaflets from Bonny Best tomato cuttings exposed for 20 hours to solutions containing: A) Q–9.25 × 10^{-4} M 8-quinolinol; L–9.25 × 10^{-4} M lycomarasmin; 3–3.08 × 10^{-4} M ferric sulfate; 2–4.62 × 10^{-4} M ferrous sulfate; B) C–9.25 × 10^{-4} M cupric sulfate; L–9.25 × 10^{-4} M lycomarasmin; 3–18.5 × 10^{-4} M ferric sulfate; 2–18.5 × 10^{-4} M ferrous sulfate.

were not exposed to lycomarasmin. The leaves of cuttings exposed to lycomarasmin and iron were withered while only a few scattered lesions were found on plants exposed to lycomarasmin alone or to lycomarasmin and iron to which 8-quinolinol had been added. Thus, at pH 6, approximately the pH of tracheal fluid, an iron chelating agent, 8-quinolinol, obviously inhibited lycomarasmin when iron was limiting.

Chelation by lycomarasmin. — From its chemical structure lycomarasmin can be predicted to form chelate complexes. That it does chelate was detected by a test for coordinate bond formation involving potentiometric titration. First, 0.5 × 10^{-4} mole of ferrous sulfate; then, 10^{-4} mole of lycomarasmin; and finally a mixture of these quantities of each were titrated with .076 N sodium hydroxide. Chelate complex formation is indicated by the liberation of hydrogen ions from lycomarasmin as chelation occurs. For this reason the titration curve for the mixture does not follow the curves for either of the constituents, but requires a greater amount of base than either constituent to raise the pH (Figure 2). Further, although a precipitate of iron hydroxide was evident at pH 5.3 when ferrous sulfate alone was titrated, no precipitate was formed even at pH 11 when the complex was titrated.

Corresponding amounts of cupric sulfate and a mixture of cupric sulfate and lycomarasmin were titrated. The results, Figure 3, indicate similarly that a chelate complex is formed between lycomarasmin and cupric ion.

The structure of lycomarasmin, together with the above evidence for coordinate bond formation, indicates that lycomarasmin forms chelate complexes with metals. Direct evidence for this and for the position of the chelate linkage is shown by other properties of lycomarasmin. On heating, it loses both toxicity and iron-chelating ability while the molecule loses the amide N as ammonia (4). Therefore chelation by lycomarasmin probably involves coordinate bonding to the amide N and electrovalent bonding to the adjacent carboxyl group of the asparagine moiety (4).

Lycomarasmin inhibition by copper ion.—The che-

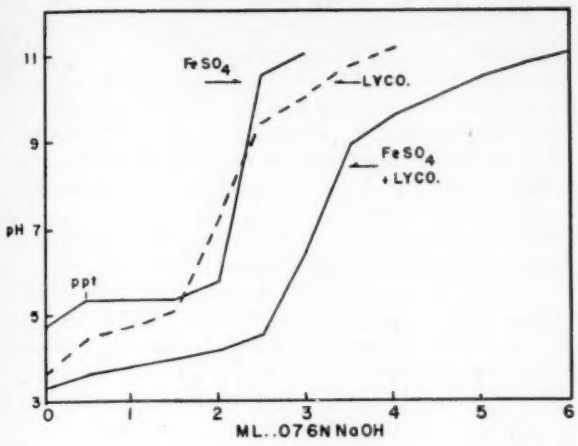

FIG. 2. Titration of 10^{-4} mole of lycomarasmin, 0.5×10^{-4} mole of ferrous sulfate, and a mixture of the 2 with 0.5 ml increments of .076 N sodium hydroxide.

lating ability of lycomarasmin has been established. If iron-lycomarasmin is less stable than copper-lycomarasmin, the quantity of the former will be decreased significantly when copper ions are introduced into a system where lycomarasmin is limiting. Therefore, toxicity will be inhibited as the quantity of iron-lycomarasmin is decreased if lycomarasmin acts as the chelate complex.

To assure that lycomarasmin would be limiting in the test of copper ion, the mixtures whose final lycomarasmin concentration were 9.25×10^{-4} M had ferrous or ferric ion concentrations of 18.5×10^{-4} N. One hour after the lycomarasmin and iron were mixed, copper sulfate was added to ½ the mixtures to give a final concentration of 9.25×10^{-4} N. The blue copper-lycomarasmin was evident in all mixtures containing the constituents whether iron was present or not. Two cuttings were then placed in each mixture.

After 20 hours all leaves of all plants placed in iron-lycomarasmin were severely injured (Figure 1). In contrast, plants placed in iron-lycomarasmin to which copper had been added were identical with the checks (Figure 1). The cuttings in each mixture had taken up 5-8 ml. Uptake was not correlated with injury. The cuttings placed in solutions of iron and/or copper showed no injury. Cuttings placed in lycomarasmin alone or lycomarasmin and copper-lycomarasmin were also identical with the checks in water.

Sixty-six hours after the cuttings were placed in the solutions, no injury was evident on any cuttings which were not exposed to lycomarasmin. The leaves of cuttings exposed to lycomarasmin and iron were withered, whereas only a few, scattered lesions were found on plants exposed to lycomarasmin and iron to which copper had been added. Evidently, 1) lycomarasmin acts as the iron-lycomarasmin chelate-complex and not as lycomarasmin alone, and 2) copper-lycomarasmin is more stable than iron-lycomarasmin; hence, copper ion inhibits lycomarasmin when lycomarasmin is limiting in a solution at pH 6.

Attempted chemotherapy of Fusarium wilt by lycomarasmin inhibitors.—The activity of an enzyme requiring a given element as a prosthetic group can be decreased by decreasing the supply of that element available to the plant (2). Similarly, the lycomarasmin inhibitors demonstrated above should decrease any activity of lycomarasmin in Fusarium wilt production by decreasing the amount of iron-lycomarasmin formed. In this way we should be able to tell what part of the wilt symptoms are due to lycomarasmin, and if the portion is considerable, discover chemotherapeutants for the disease.

The effectiveness of the inhibitors as chemotherapeutants was tested in the following manner. Five of the 10 tomato plants in each of 5 replicates were inoculated. After 19 days foliar symptoms of wilt began to appear on the inoculated plants and were scored.[3] From each replicate 2 inoculated and 2 check plants were placed in individual flasks of distilled water. An inoculated and a noninoculated plant were placed in individual flasks containing 6.2×10^{-5} M cupric sulfate, 6.9×10^{-4} M 8-quinolinol, or 3.3×10^{-4} M ferrous sulfate. These concentrations of the compounds had been found to cause slight injury, indicating that they were taken up, but not such severe injury that the effects of lycomarasmin would be obscured. After 6 days foliar symptoms were again scored (Table 1).

The change in foliar score of diseased plants set in water for 6 days was .40 greater than the corresponding change for healthy plants. If the lycomarasmin caused a considerable portion of the change in the diseased plants, the relative change of score should be less than .40 for plants set in the inhibitors. The relative change of score for cupric sulfate was .32, not significantly less than water. From this the inference is drawn that 1) lycomarasmin is not limiting and therefore copper ion is not a suitable inhibitor, or 2)

[3] Scoring methods are described by Dimond, et al (3).

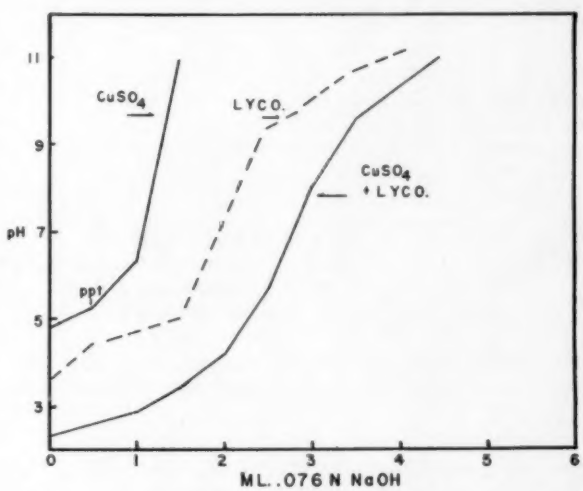

FIG. 3. Titration of 10^{-4} mole of lycomarasmin, 0.5×10^{-4} mole of cupric sulfate, and a mixture of the 2 with 0.5 ml increments of .076 N sodium hydroxide.

TABLE 1.—*Change in foliar score[a] for tomato plants after being in solutions for 6 days*

Solution	Inoculated plants	Uninoculated plants	Relative change[b]
Water	1.03[c]	0.63	0.40
$CuSO_4$, 6.2×10^{-5} M	1.28[d]	0.96	0.32
8-Quinolinol, 6.9×10^{-4} M	1.24	1.38	—0.14
$FeSO_4$, 3.3×10^{-4} M	1.16	0.88	0.28

[a] Score ranges from 0 for normal to 4 for abscinded leaf. Mean score on noninoculated plants was .08 and inoculated plants was 0.98 when plants were placed in solutions.
[b] Standard error of difference between water and other treatments was 0.31.
[c] Mean of 10 plants, 5–8 leaves per plant.
[d] Mean of 5 plants.

lycomarasmin does not cause a considerable portion of the change in foliar score in the diseased plant.

The relative change of score for 8-quinolinol was —0.14. The difference between this change and the change for water is significant at the 10 per cent point. If this difference is not due to chance, the inference would be drawn that iron is limiting and 8-quinolinol is a suitable inhibitor. However, in similar experiments of the same design, the difference between water and 8-quinolinol has been nil. Therefore, we conclude that the difference between the relative changes for water and for 8-quinolinol, which is not highly significant, is due to chance. This conclusion is consistent with the observation that the relative change of score for ferrous sulfate is no greater than the change for water. Also it is consistent with Scheffer's (8) observation that iron deficient plants develop as severe symptoms of Fusarium wilt as do normal plants.

The failure of ferrous sulfate to increase the development of Fusarium symptoms is particularly difficult to reconcile with the hypothesis that lycomarasmin plays an important role in the development of these symptoms. Evidence presented above indicates that lycomarasmin acts as the iron complex. The well-known fact that lycomarasmin is potentiated by iron must mean, therefore, that the quantity of iron available for combination with lycomarasmin in the plant is insufficient to permit maximum damage by lycomarasmin. Consequently, the failure of ferrous ion to increase the severity of wilt symptoms when it is added to diseased plants may mean that lycomarasmin is not important in Fusariuam wilt development.

Compounds that inhibit lycomarasmin when lycomarasmin or iron are limiting and iron, which increases lycomarasmin toxicity when iron is limiting, did not effect the development of Fusarium wilt symptoms. Therefore, we cannot say that lycomarasmin produces an important part of Fusarium wilt symptoms.

SUMMARY

Lycomarasmin is known to be 10 times as toxic to tomato cuttings if applied together with iron rather than alone. When 8-quinolinol, which forms a stable chelate complex with iron, is added to a mixture of lycomarasmin and a limited amount of ferrous or ferric ion, lycomarasmin toxicity to tomato cuttings is decreased. When cupric ion, which forms a chelate complex with lycomarasmin, is added to a mixture of ferrous or ferric ion and a limited amount of lycomarasmin, lycomarasmin toxicity is decreased. Therefore, the lycomarasmin complex with iron must be less stable than the lycomarasmin complex with copper. Further, lycomarasmin must act as the iron complex. The 2 lycomarasmin inhibitors, 8-quinolinol and copper ion, did not retard the development nor did iron ion speed the development of foliar symptoms of Fusarium wilt of tomato. Therefore, lycomarasmin cannot be said to cause an important part of the symptoms of Fusarium wilt.

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THE PERFECT STAGE OF COLLETOTRICHUM PHOMOIDES ON TOMATO [1]

GEORGE SWANK, JR. [2]

The fungus causing tomato anthracnose was first reported and described by Saccardo (6) in Italy in 1878, and named *Gloeosporium phomoides*. In 1884, Arthur (1) reported the occurrence of the fungus in New York, and in 1891 Chester (2) reported an organism similar to that described by Saccardo and named it *Colletotrichum lycopersici* n. sp. In a latter publication Chester (3) recognized that setae may be present or absent and on this basis renamed the causal agent *Colletorichum phomoides* (Sacc.) Chester. The writer has found no previous account of a sexual stage of this organism.

ORIGIN OF STRAIN.—During the course of investigations of the variability of monosporous isolates of *C. phomoides*, a strain of the fungus was found which formed perithecia, asci, and ascospores in culture. (Fig. 1, A.) A tomato fruit had been inoculated with a monoconidial, imperfect isolate, and typical anthracnose lesions developed. All monoconidial reisolates obtained were similar in cultural characteristics but produced perithecia, asci, and ascospores as well as conidia in culture. The tomato from which the strain was isolated apparently had not been contaminated from outside sources. It had been inoculated as follows: the colony arising from a single conidium was placed on a tomato fruit in a drop of sterile water. A dissecting needle was used to wound the fruit at the place of inoculation, then a moist sterile cotton pad was placed over the area of the fruit inoculated for a period of 48 hours. The tomato fruit was kept in a previously sterilized moist chamber. The tomato fruit had been surface sterilized in bi-chloride of mercury 1: 1000 and rinsed in running tap water before inoculation. The technique used in reisolating was a modification of that described by Hanna (4). Later single ascospores were also isolated with the use of a micromanipulator.

VERIFICATION OF PATHOGENICITY.—Cultures arising from single ascospores were used to inoculate tomato, bean, pepper, and apple fruits. Lesions developed on all. Those that developed on the tomato fruits were similar in appearance to those resulting from inoculations with known conidial strains of the anthracnose organism. (Fig. 1, C). They were sunken and circular, with alternating concentric zones of light and dark color beneath the epidermis. Many acervuli developed in the lesions. Lesions that developed on apple fruits were light brown, soft, and later became watery. The reisolations from the tomato fruit lesions were culturally the same as the original isolate used as inoculum, including the production of perithecia, asci, and ascospores. The perfect stage was not observed in the diseased tomatoes or apples.

A suspension from a monoascosporus culture containing ascospores and conidia was atomized on the foliage of 6-in. Rutgers tomato plants. After inoculation the plants were placed in a constant temperature and humidity chamber for 24 hours. The temperature was maintained at 26° C. and the humidity at approximately 100 per cent. Five days later, small, circular, brown, necrotic lesions appeared on the foliage. Dieback and coalescing of lesions at the tips of leaflets was quite common.

It was concluded that a mutation of an imperfect strain of the anthracnose fungus occurred in the tomato fruit that resulted in the origin of a strain which produced perithecia, asci, and ascospores in culture and causes anthracnose symptoms on tomato fruit and foliage.

EXAMINATION OF SEXUAL STAGE.—Tomato stems were cut into small sections and placed in test tubes containing moist cotton pads, then autoclaved at 15 lb. pressure for 30 minutes. Conidia from monoascosporus cultures were placed on the stems. Perithecia, asci, and ascospores developed 2 weeks later. (Fig. 1, B). Sections about 0.5 cm. long were cut and placed in F. A. A. (5 ml. of formalin, 5 ml. of glacial acetic acid, 90 ml. of 50 per cent ethyl alcohol), fixation solution for a period of 24 hours, then in a series of alcohol solutions of 60, 70, 80, 95 and 100 per cent followed by immersion in toluene series of 25, 50, 75, and 100 per cent. Liquid paraffin was added to the toluene and sections while they were in an oven at 40° C. Three changes of liquid paraffin were made before mounting the sections. Serial sections 11 microns in thickness were cut on the rotary microtome and fixed on the slides with Haupts' fixative. The stain used was iron-alum haematoxylin described by Rawlins (5).

MORPHOLOGY.—In culture, on 2 per cent potato-dextrose agar, the mycelium grows rapidly, white when young, dark grey with age; perithecia occur singly or in groups, form 14–18 days after transfer; they are irregular, 130–242 μ long $\times$ 93–180 μ in diameter; the ostioles are beaked, hairy, walls brownish black and membraneous. Asci 56–112 μ long, hyaline, 8 spored; ascospores oval to eliptical, non curved, hyaline, uninucleate, 15 $\times$ 19 μ long $\times$ 5–7 μ wide; (Fig. 1, E), ascospores form 1–2 septa at germination. Hosts: *Lycopersicon esculentum* Mill.; *Malus malus* (Tourn) Hill; *Phaseolus vulgaris* L.; *Solanum piperatum* L.

Latin Description.[3]—***Glomerella phomoides*** Swank sp. nov. Mycelio in culturis rapide crescenti, juveni

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[3] The author is indebted to Edith K. Cash, Division of Mycology and Disease Survey, United States Department of Agriculture, for the preparation of the Latin description.

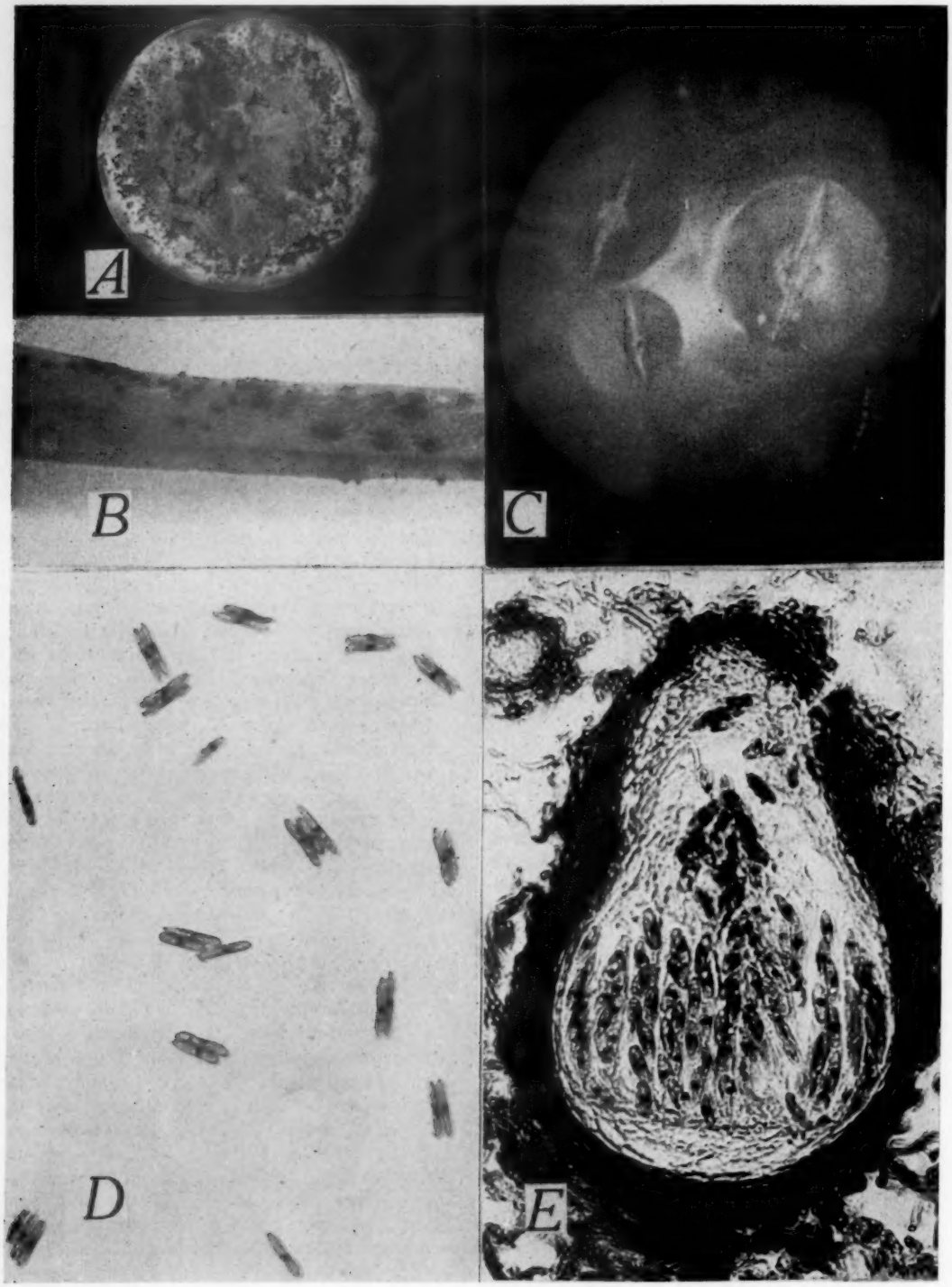

FIG. 1. *Glomerella phomoides* Swank sp. nov. A) culture growing on 2 per cent potato-dextrose; B) perithecia formed on a tomato stem; C) lesions on a tomato fruit; D) uninucleate conidia, 480×; E) perithecium, asci and uninucleate ascospores sectioned at 11 microns thickness, 480×.

albo, in aetate opaco-griseo; peritheciis singulis vel aggregatis, dies 14–18 post translationem efformatis, irregularibus, 130–242 μ longis, 93–180 μ in diam.; ostiolis rostratis, crinitis, brunneo-nigris, membranaceis; ascis 56–112 μ longis, hyalinis, octosporis; ascosporis ovato-ellipticis, rectis, hyalinis, uninucleatis,

15–19 × 5–7 μ, ad germinationem 1–2-septatis. Conidial stage: *Colletotrichum phomoides* (Sacc.) Chester. (3)

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A PHYTOPATHOLOGICAL NOTE

Proof that the Fungus Pads on Oak Wilt-killed Trees Are a Growth Form of Endoconidiophora Fagacearum.[1] Bert M. Zuckerman and E. A. Curl.

Although it has been assumed quite generally that the thick pad,[2, 3] or cushion,[4] found almost always in the center of the subcortical mat of the oak wilt fungus is a special structure of the fungus, doubt that the pad does belong to *Endoconidiophora fagacearum* Bretz has been expressed. Supporting evidence on both sides of the question is, at present, largely circumstantial.

Curl, Stessel, and Zuckerman[2] showed that tissue from the interiors of pads, when transferred to potato-dextrose agar, frequently produced pure cultures of *E. fagacearum*. Occasionally, pads were found that were not surrounded by endoconidium-bearing mats of the oak wilt fungus. Tissue taken from the interiors of these pads also gave pure cultures of *E. fagacearum*.

The production of pads on a solid culture medium was reported by Curl, Stessel, and Zuckerman.[2] Cultures of known pathogenicity from single spore isolates were used in the work. When the fungus was grown on chestnut meal agar (20 gm. ground Chinese chestnut meats and 20 gm. agar per liter) at 25°C for 5 weeks and then placed at 8°C, the pads developed in the central portions and near the outer edges of the cultures. Pads also developed in cultures kept continuously at 25° and 16°C.

Recently the fungus was grown on a liquid medium (20 gm. ground Chinese chestnut meats in 100 ml. water) in 200 ml. Erlenmeyer flasks. When grown without shaking, the fungus formed thick pads that were circular in outline, were partially submerged, and were covered by a sparse to dense mycelium. When grown in shaken flasks, it did not produce a solid pad but produced, in addition to conidia, large numbers of cells similar to those that make up the pad; these cells were dispersed singly or in clusters of 2–4 throughout the culture.

Pads found in nature and those produced on liquid and solid chestnut media were similar in color and texture. The daedaloid surface pattern and the deep rifts characteristic of pads formed under natural conditions were observed in pads produced on artificial media.

Sections of pads found in nature showed that the pads were composed of thin-walled, inflated, irregularly shaped or ovoid to spheroidal cells that, when young, were filled with a greenish, homogeneous cytoplasmic mass. These cells varied in size from 13 × 16μ to 40 × 62μ. Numerous tan hyphae ramified among the cells, often swelling to form large cells that merged with the other large cells that composed the pad. The cell contents disappeared as the pad aged. Cells that composed young and old pads produced *in vitro* (Fig. 1, A) were morphologically similar to the cells that composed pads formed in nature (Fig. 1, B).

Young pad cells were separated by a streak-dilution technic and single cells were transferred to potato-dextrose agar plates. Three of 9 cells so transferred gave rise to typical *E. fagacearum* cultures. Pathogenicity of these cultures was tested and found positive. It may be concluded that young pad cells are capable of growth, despite the remote possibility that a spore had, in each of the 3 cases, adhered to the cell wall and was not seen when the cell was examined.

The genesis of pad cells from hyphae was studied in laboratory cultures. In one mode of pad cell formation, individual cells in a hypha swelled (Fig. 1, C), broke off from the rest of the filament (Fig. 1, D), and merged with cells that had been formed previously. The development of pad cells in this manner was observed in the following experiment. Hyphae from a liquid culture were placed on a cover slip that had been coated with a thin layer of potato-dextrose agar. The cover slip was placed, coated surface down, on a deep well slide and sealed at the edge with agar. The hyphae were observed at a magnification of 440 diam-

[1] This work was supported in part by grants from the Forest Preserve District of Cook County, Illinois, and the National Oak Wilt Research Committee.

[2] Curl, E. A., G. J. Stessel, and B. M. Zuckerman. 1953. Subcortical mycelial mats and perithecia of the oak wilt fungus in nature. Phytopathology 43: 61-64.

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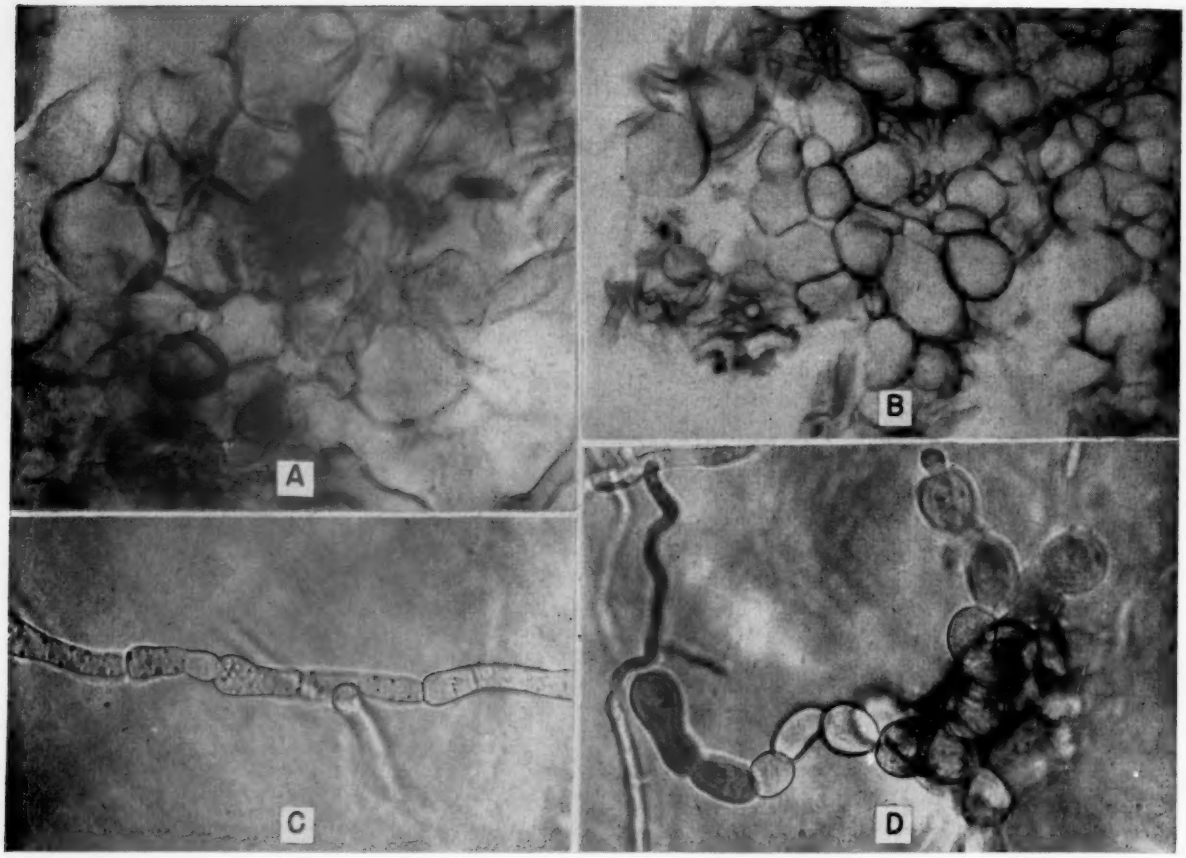

Fig. 1. A) Freehand section of pad produced on chestnut meal agar, showing typical cells, 270×; B) microtome section of naturally occurring pad, showing typical cells and hyphae, 180×; C) early stage of hyphal segmentation, 180×; D) advanced stage of hyphal segmentation, showing fully formed young pad cells, 180×.

eters. Where septations occurred, the cells assumed an ovoid to elliptical shape during an interval of 24–30 hrs. and thereafter became detached from the hyphal strands.

In summary, the following facts are submitted as new evidence that pads generally associated with subcortical mats of *E. fagacearum* in nature are a form of the fungus: 1) when the fungus was grown in a liquid medium, pads morphologically similar in macroscopic and microscopic characteristics to those fund in nature were formed; 2) the genesis of pad cells from hyphae was observed; 3) single pad cells gave rise to cultures of *E. fagacearum*.—Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Urbana, Illinois.

REPORT AND ABSTRACTS OF THE 1953 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Southern Division, American Phytopathological Society, held its 1953 Annual Meeting on February 9, 10, and 11, 1953, in New Orleans, Louisiana, in conjunction with the 50th Annual Convention of the Association of Southern Agricultural Workers. Approximately 120 members and guests attended. A total of 45 papers were presented. Two symposia and 1 conference were held. The symposia dealt with 1) Teaching Plant Pathology in the South, presided over by G. F. Weber of Florida, and 2) Problems Connected with *Sclerotium rolfsii*, conducted by W. M. Epps of South Carolina. A conference on Stone Fruit Viruses was presided over by L. W. Boyle of Georgia. The Cotton Disease Council also met in conjunction with the Southern Division on February 11, 1953.

J. G. Dickson, National President, honored the Southern Division with his attendance at its meetings. He spoke informally to the members during their annual business meeting on February 10, 1953. The Southern Division was also honored by the presence of J. H.

Jensen and G. F. Weber, Vice-President and President Elect, respectively, of the parent Society.

At the annual business meeting officers elected for 1953 were: *President* (1 year) S. S. IVANOFF; *Vice-President* (1 year) J. H. MILLER; *Councilor* (2 years, expires 1955) S. J. P. CHILTON; *Secretary-Treasurer* (3 years, expires 1954) J. A. LYLE.

JAMES A. LYLE, *Secretary-Treasurer.*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Evidence for the occurrence of a hitherto unrecognized growth-retarding disease of sugarcane in Louisiana. ABBOTT, E. V. During prolonged periods of dry weather, which have occurred frequently during the growing season in the sugarcane district of Louisiana in recent years, marked growth retardation and stunting of plants of some varieties were observed. In general, the symptoms exhibited by affected plants were similar to those of a virus disease of sugarcane described recently in Australia: growth retardation, deficient root system, reduction in number and diameter of stalks, often paler color of the leaves, and frequently an orange-yellow discoloration of the vascular bundles at the nodes of mature stems. In October 1951, juice was extracted from the stems of plants of 2 varieties showing these symptoms and inoculated into stem cuttings from vigorously-growing and apparently healthy plants. These were planted in the field with appropriate controls. Results obtained in 1952 showed that growth and weight of cane from inoculated cuttings were substantially reduced to a degree comparable with that of the plants from originally stunted cane, as compared with healthy controls, and other symptoms observed in the field in stunted plants were evident.

A Sclerospora infection of sugarcane in Louisiana. ABBOTT, E. V., AND C. A. SCHEXNAYDER. A disease of sugarcane caused by a species of *Sclerospora* was identified in Louisiana in 1952. Affected plants are severely stunted, show excessive tillering, and often consist of a cluster of stunted shoots resembling a tuft of grass. Usually only a few or no jointed stalks are produced and these may or may not be infected by the fungus. Root development is very deficient. The leaves are thickened, abnormally erect, and show chlorotic markings somewhat similar to the mottling of mosaic. On older leaves these may become reddish brown in color. Stem galls occur commonly, generally at or near the ground level. These vary greatly in size and shape and at times develop into adventitious buds which in turn give rise to shoot proliferation. Oospores are produced abundantly in the leaf tissues, but the asexual stage has been observed infrequently. The disease is similar to and perhaps the same as a Sclerospora infection of sugarcane recently reported from Queensland, Australia, and one observed by the senior author in Peru.

Effectiveness of various field inoculation procedures in testing tobacco for black shank resistance. APPLE, J. L., AND G. B. LUCAS. Over a 3-year period various types of field inoculation of tobacco plants were made with the black shank fungus (*Phytophthora parasitica* var. *nicotianae*) in an effort to find a standard technique to determine the levels of resistance of different breeding lines. Three kinds of inoculum were used: 1) pure cultures of the fungus grown on sterilized oat grains, 2) aqueous suspensions of zoospores, and 3) infected plant material. Either root or stem inoculations were made on a 1) susceptible variety, 2) a moderately resistant variety, and 3) a highly resistant variety. Inoculations were made at transplanting time or on various dates thereafter. Results indicated that stem inoculations are not practical for large scale field tests with present breeding stocks. Plant material inoculum did not separate moderate from highly resistant lines, it was not uniform in quality, and was not always available at transplanting time. Zoospores are delicate and required optimum moisture and temperature conditions for successful infection. Root inoculations with pure culture oat inoculum at transplanting time, made under conditions of ample soil moisture and warm temperatures (ave. 68° F.), were the most effective in separating moderately resistant from highly resistant lines.

Parasitic nematodes associated with diseased roots of sugarcane. BIRCHFIELD, WRAY. Large numbers of nematodes of unidentified species of *Tylenchorynchus* and *Pratylenchus* were found associated with abnormal sugarcane roots in Louisiana. Symptoms of the disease were dwarfing of the entire plant, apparently resulting from an impaired root system. The diseased roots lacked the normal fine network of feeder roots. The small roots were blunt and coarse, showing characteristic browning and lesions. To determine the role of the 2 kinds of nematodes as causative agents of the disease they were collected for inoculation purposes from soil and roots of sugarcane growing in the field. After sieving, the nematodes were separated further by means of the Baermann funnel technique. Twelve 8-in. clay pots containing steam-sterilized soil were planted with the C.P. 29/116 variety of sugarcane. In each of 6 pots over 1400 nematodes were added by pouring a suspension of the nematodes in distilled water over the emerging sugarcane roots. The remaining 6 pots served as checks. The characteristic disease was reproduced in the inoculated series while the checks remained free of the disease. Nine weeks after inoculation both kinds of nematodes had increased in number in the inoculated series.

Virulence of a root pathogen as conditioned by nutrition. BOUCHEREAU, PAUL E. Pythium isolates obtained from sugarcane varieties growing on virgin soil generally were found to be only mildly parasitic on corn roots. When these isolates were repeatedly transferred on salicylic aldehyde media of 25 p.p.m. concentration, some of the treated isolates were found to have a greater ability to produce injury to the corn plants than either the original isolates or the aldehyde acting alone. The more pronounced effects were produced with those isolates which originally occupied a median position of relative virulence. That the effect of the aldehyde was on the organism rather than on the host was indicated by tests with sugarcane varieties of varying degrees of resistance to root rot. Flats of sterile sand were infested with the normal and aldehyde-treated isolates. Seed pieces were planted immediately but sufficient time elapsed before germination to allow complete oxidation of the aldehyde. The greater reduction of root weight produced by the aldehyde-treated isolates as compared with the effects on sugarcane of the non-treated isolates, was interpreted as an indication of increased virulence. Chemical analyses of the expressed juice of resistant and susceptible sugarcane varieties provided evidence indicating that the degrees of damage produced by even virulent strains may be limited by the nutritional compounds contained in the roots themselves.

Variation in the pathogenicity of Sclerotium rolfsii Sacc. isolates on peanuts. COOPER, W. E. The range in pathogenic variability of 52 isolates (39 tested in 1950 and 43 tested in 1951) was studied. Most isolates were obtained from peanuts during 1948 and 1949 and were stored under refrigeration. Inocula, prepared by growing isolates on steamed oats for approximately 2 weeks, were carefully scattered on the ground under peanut plants during midsummer at a rate of approximately 250 gm. per 20-ft. row. Pathogenicity was based on the number of dead stems counted at the soil surface. Each year the different isolates ranged from very weakly to very strongly pathogenic.

The relative pathogenicity of 26 of the 31 isolates included in both years was the same although 5 isolates were much less pathogenic the second year. In 1952, 5 weakly, 5 moderately, and 5 strongly pathogenic (1951 results) isolates were compared. The weakly pathogenic isolates killed few stems, the intermediate group varied somewhat, and 3 of the pathogenic group remained strongly pathogenic, whereas 2 isolates had reduced pathogenicity.

Resistance and susceptibility to root-knot nematodes in tomato and sweet potato. DEAN, JACK L. AND F. BEN STRUBLE. Plants were inoculated with the root-knot nematode (*Meloidogyne incognita*) by a method which makes it possible to determine the time of entry of the nematodes within the limits of whatever period is chosen for inoculation. Root systems were stained and examined microscopically at various time intervals after inoculation. Root systems of resistant tomatoes, *Lycopersicon peruvianum* and *L. peruvianum* hybrid, were invaded by fewer larvae, usually half or less, than those of susceptible Marglobe tomatoes. Nematodes entering resistant roots produced extensive necrosis of host tissue within 48 hours. Two weeks after inoculation most of the invading larvae had died and disappeared. No larvae in resistant roots ever developed as far as the second molt. No differences related to resistance and susceptibility were noted between sweet potato varieties with respect to the number of larvae entering the roots. Nematodes entering resistant sweet potato roots (Orlis, Oklahoma 46, Oklahoma 29) produced necrosis of host tissue several days after inoculation. Necrosis was not observed in susceptible Allgold roots. A few nematodes in all lines of resistant sweet potatoes developed to egg-laying maturity but most larvae died and disappeared before reaching that state.

A Re-evaluation of oat seed treatment. EARHART, R. W. In our studies of seed treatment for controlling oat diseases, we measured the effectiveness of several different materials. Special emphasis was placed on protection of seedlings from infection, ease of application, time of treatment, and effects of storage. On the basis of these tests, only 2 materials, Ceresan M and Panogen, can be recommended for our conditions.

A Rhizoctonia foliage blight of cucumber. ELLIS, D. E., AND L. H. PERSON. A striking blight was observed on the foliage of Model variety cucumber in 1 field in Warren County, North Carolina, in July 1952. The disease was limited to a poorly-drained portion of the field. From a distance the plants exhibited a whitish cast suggestive of a leaf scald. Close examination revealed definite leaf lesions ranging from small, pale-green, water-soaked spots to large lesions up to 2 in. in diameter with white centers and pale-green margins. Microscopic examination and isolations showed that *Rhizoctonia* sp. was consistently associated with the disease and its pathogenicity was established by inoculation tests in the greenhouse. In pure culture the fungus resembled closely isolates of the *Corticium microsclerotium* type from certain other hosts with which it was compared. In cross inoculation studies isolates that were morphologically alike from cucumber, snap bean, and Ladino clover caused similar symptoms on cucumber foliage. Furthermore, isolates from these 3 hosts caused typical web-blight symptoms on Tendergreen variety snap bean.

Reaction of sweet potato varieties and seedlings to internal cork disease. FEAZELL, GEORGE D. In determining the response of varieties and seedlings of sweet potato to the internal cork disease, the following technique was used: mother potatoes of the variety or seedling were core-grafted with a core from corky Porto Rico potatoes; slips from the core-grafted mothers were planted in the field, and crops were harvested from these. Some of the roots were cut and examined at harvest time, and the remaining roots were stored at 70° to 80° F. for about 7 months, then examined. The percentage of affected potatoes and the degree of damage were recorded. Based on the above results, the varieties were rated as follows: *Susceptible,* Porto Rico Unit 1, Queen Mary, Goldrush; *Intermediate,* Heartgold; *Resistant,* Allgold, Pelican Processor. Rating of Louisiana seedlings that appeared promising as future varieties were: *Susceptible,* 9-47; *Intermediate,* L-240, 9-2, 9-39, 9-49; *Resistant,* 9-18, 9-65, 9-65A, L-224. When cores were taken from cork-free roots of Heartgold and Allgold varieties and core-grafted into cork-free Porto Rico roots, internal cork developed heavily in the Porto Rico roots. This indicates that roots of Allgold and Heartgold may carry the infective agent of internal cork without expressing corkiness, even after 7 months in storage.

Susceptibility to tomato buckeye rot fungus in Tennessee. FELIX, E. L. Wide range of susceptibility to *Phytophthora parasitica* var. *terrestris* is indicated further by natural and artificial inoculations, which in general gave similar results, and by cultural study. All species of tomato (U.S.D.A. and other lots), so far tested, are susceptible to buckeye rot. Fruits of the following plants, at least in some collections, are highly susceptible: *Lycopersicon cerasiforme, L. esculentum, L. glandulosum, L. hirsutum* var. *glabratum, L. peruvianum, L. peruvianum* var. *dentatum* and *L. peruvianum* var. *humifusum; Nicandra physalodes* (induced); *Physalis peruviana* and *P.* spp.; and *Symphoricarpos albus* var. *laevigatus. Clarkia elegans* and *Rheum rhaponticum* plants succumb readily. Moderate to high resistance occurs in fruits of *L. pimpinellifolium; Physalis inoxcarpa, P.* spp.; and *Solanum capsicastrum*; and high resistance in *Solanum carolinense, S. integrifolium,* and *S. nigrum* inoculated by the 2 methods. Wonderberry (*S. nigrum* var. *guineense*) apparently is immune and infection of *S. tuberosum* or *Ricinus communis* has not been observed. Fruits of *Capsicum frutescens* var. *grossum* (California Wonder) and *S. melongena* var. *esculentum* (Black Beauty) are rarely affected under local field conditions. Absence of tobacco black shank in fields heavily infested with the buckeye rot fungus indicates causal differences, though tomato fruit rot can be induced by the tobacco black shank fungus.

Organic fungicides in relation to brown rot control on peaches. FOSTER, H. H. Six spray treatments were compared in randomized single tree plots of Shippers Late Red variety, replicated 7 times. All the plots were treated with Parathion at the rate of 1½ lb./ 100 gal., as an insecticide. Control plots received 7 applications of the insecticide while plots receiving the 5 fungicide treatments were given 8 fungicide sprays. Materials used are listed in decreasing order of control of the brown rot fungus (*Monilinia fructicola*): 1) Wettable sulfur, 6 lb./100 gal.; 2) Vancide 51, 3 pints/ 100 gal. for the first 5 sprays followed by wettable sulfur for the remaining 3 applications; 3) Manzate 1½ lb./100 gal.; 4) General Chemical Bis (p-Nitrophenol) carbonate, 2 lb./100 gal.; 5) Penn Salt NP 770, 2 lb./100 gal.; 6) Control. All applications were made with a power sprayer. The first spray was applied at petal-fall. Peaches were harvested 3 weeks after the final fungicide application and data were recorded after a 4-day storage period at room temperature (approximately 80°–90° F.). Severe foliage injury developed following the use of Vancide 51. The deepest red color developed on Manzate sprayed peaches.

The influence of greenhouse temperatures on the development of Erysiphe graminis tritici on wheat seedlings. FUTRELL, MAURICE C. The response to *E. graminis tritici* was studied on 15 spring wheat varieties grown in the greenhouse at temperatures of 16°, 20°, 24°, and 28° C. during the late winter at Madison, Wisconsin. Seedling plants were dusted with conidia in the 2-leaf stage. Susceptible varieties showed maximum infection at 20° C.; however, infection was only slightly less at 16° and 24° C. Sturgeon (C. I. 11703) was more susceptible on the leaf sheath than on the leaf blade, reaching maximum infection on the sheath at 20° C. Egypt (C. I. 12100) showed a low percentage of type 4 infection reaching a maximum on the leaf blade at 24° C. The powdery mildew reaction of Hope (C. I. 8178) responded more to temperature than did that of other varieties. Leaf blades of Hope were resistant at 16° and 20° C. but were moderately susceptible at 24° C. Development of mycelium on the leaf sheath of Hope seed-

lings was much less at 16° and 20° than at 24° C. Khapli (C. I. 4013), *Triticum timopheevi* Zhuk. and selections C. I. 12632, and C. I. 12633 from hybrids with the latter showed high resistance to the fungus at all temperatures.

The sting nematode, Belonolaimus gracilis, on cotton and other crops in South Carolina. GRAHAM, T. W., AND Q. L. HOLDEMAN. Careful studies of the sting nematode and its pathological effects on various crops were made during 1950-1952 in South Carolina. Severe field damage occurred on cotton, corn, soybeans, cowpeas and strawberries. Field symptoms were retarded growth and decayed roots but they were not distinct from the symptoms of other nematode diseases like the meadow nematode root rot. Symptoms on the above crops were reproduced in the greenhouse. Inoculation trials were made with singly-picked nematodes placed in autoclaved soil where subsequently cotton showed retarded growth and root decay symptoms associated with high populations of sting nematodes. Basic technic in establishing the parasitic relation of this nematode was the modified Baermann funnel technic used to screen soil samples. As this nematode is an external feeder it was not found inside the roots but only in soil samples. Soil fumigation experiments in the greenhouse and the field gave control of nematodes and restored normal crop growth to infested soil. Ethylene dibromide and DD mixture were effective at rates of 4 and 20 gal. per acre or more, respectively.

A new race of Fusarium causing wilt of cowpea. HARE, W. W. In developing a breeding program in Mississippi for incorporating resistance to Fusarium wilt in preferred varieties of cowpeas, isolates representing the 2 races of *F. conglutinans* Wr. described by Armstrong were used. A collection of isolates from the state was made. With appropriate varieties inoculated as described previously, these could be classified as race 1 or race 2. Groit was killed by race 1 and Extra Early Blackeye was not. Extra Early Blackeye was killed by race 2 and Groit was not. However, certain isolates classified as race 2 were more damaging to varieties partially resistant to race 2. The Arlington variety was killed by these isolates. This variety was not killed by race 1 or race 2. The reaction of Groit and Extra Early Blackeye to these isolates was the same as to race 2. Similar isolates were collected from several widely separated areas in Mississippi. On the basis of the greater severity on varieties partially resistant to race 2 and the differential reaction of the variety Arlington, it is suggested that such isolates be designated race 3 of *Fusarium conglutinans*.

Seed treatments and soil drenches for the control of damping-off of tomatoes. HARRISON, A. L. Orthocide 406 (50%), Carbide and Carbon Chemicals Company 5400, and Aagrano-350 constantly have given as good or better control of pre-emergence damping-off of tomatoes as N. I. Ceresan, 2% Ceresan, and Cuprocide when used as seed protectants in soil artificially infested with oat cultures of *Pythium* sp. and *Rhizoctonia solani*. Some 25 different chemicals were used in these tests. Orthocide 406 and C.C.C. 5400 were used at rates ranging from 4 oz. to 24 oz. to 100 lb. of seed without signs of phytotoxicity. Aagrano-350 caused some injury at high dosage rates. The zinc salt of Vancide 51 has, in preliminary tests, indicated that it also is a promising seed protectant for the control of pre-emergence damping-off of tomatoes. Orthocide 406 and C.C.C. 5400, as soil drenches just prior to seedling emergence, have helped reduce losses from post-emergence damping off. Under the same conditions Fermate, as a soil drench, gave good control but caused some injury. In split plot tests with different seed protectants and soil drenches significant interactions have been obtained, indicating that some soil drenches can be used safely with some seed protectants but not with others. Fermate has, in some tests, caused significant reductions in stand with Arasan treated seed and Vancide 51 significant reductions with both N.I. Ceresan and Cuprocide treated seed.

Results of some peanut seed treatment tests in Texas. HARRISON, A. L., B. C. LANGLEY AND G. M. WATKINS. Seed treatment tests on Spanish type peanuts in Texas from 1942-52 have demonstrated that 2 per cent Ceresan was equal or superior to the standard Arasan and Spergon peanut seed protectants ,and that Arasan was superior to Spergon. Results with Phygon were erratic, being good in the Stephenville area but poor in other parts of the State. The new fungicides, Carbide and Carbon Chemicals Company 224 and 5400, Aagrano-350, Mathieson Chemical Company 275 and Orthocide 406, as peanut seed protectants, gave significant increases in stand over untreated seed. In some tests these new fungicides gave significant increases in stand over Arasan- and Spergon-treated peanut seed. When the results of the peanut seed treatment tests were combined, according to rank in the various tests, the seed protectants were in the following descending order: C.C.C. 224, Aagrano-350, Orthocide 406, C.C.C. 5400, Mathieson 275, 2 per cent Ceresan, Arasan and Spergon. Over 30 fungicides were tried as peanut seed protectants but most of them were discarded because of phytotoxicity or ineffectiveness in controlling seed decay.

Population trends of the sting nematode under different crops in greenhouse pot culture. HOLDEMAN, Q. L., AND T. W. GRAHAM. Greenhouse studies gave preliminary information which may be of value in planning field work and rotation trials. Seeds or plants were planted in pots containing naturally infested field soil. After 60–75 days a soil sample from each pot was examined for nematode populations. The modified Baermann funnel technique was used. The sting nematode consistently increased to high populations (index value of 15 or above) on corn, oats, wheat, barley, rye, alta fescue, cowpea, soybean, summer squash, and cotton. Initial attack on sweet potato and velvet bean was so severe that populations dropped. Population build-up was somewhat inconsistent under peanut, crimson clover, ladino clover, tomato, bell pepper, cucumber, strawberry, ragweed, cocklebur, crowfoot grass, bermuda grass, and crab grass, but evidence indicates these crops increased or maintained nematode populations. The presence of larvae under a crop was regarded as evidence that reproduction occurred. No larvae were observed where nematodes consistently failed to increase under tobacco, watermelon, and crotalaria.

Breeding oats for resistance to diseases and cold. IVANOFF, S. S. Some progress has been made in breeding oats for combined resistance to Crown rust (*Puccinia coronata*), Victoria blight (*Helminthosporium Victoriae*) and cold. Sources for rust resistance have been chiefly Landhafer and Santa Fe. Strains of oats of advanced hybrid generations with certain desirable agronomic qualities are being increased. One of the few strains derived from crosses with Landhafer that show good grain characteristics, high crown-rust and Victoria blight resistance, but not much cold resistance (C.I. 5930: Victorgrain × Landhafer) is being given a regional test in the Gulf Coast area. Another approach towards obtaining resistant oats has been made by adapting laboratory mass screening methods by means of which a few disease-resistant seedlings have been isolated from among several million susceptible seeds of varieties derived from Victoria hybrids. The P.I. 174544 oat strain (C.I. 6665) once supposed to be leaf rust resistant in many parts of the United States, proved only moderately resistant in South Mississippi in 1952.

The influence of source and level of nitrogen on growth of Pseudomonas solanacearum in culture. KELMAN, ARTHUR. Five isolates of *Pseudomonas solanacearum* representing a wide range in pathogenicity were grown in a basal nutrient solution with different sources of nitrogen (potassium nitrate, sodium nitrate, ammonium nitrate, and ammonium sulfate) at concentrations ranging from 98 to 1568 p.p.m. of nitrogen. All isolates responded similarly when supplied with a given source and concentration of nitrogen. Results indicated that the isolates utilized ammonium nitrogen more readily than nitrate nitrogen. As concentrations

of nitrate nitrogen were increased above 196 p.p.m., growth levels decreased with little development at 1568 p.p.m. This inhibition of growth was not evident in solutions containing moderate to high concentrations of ammonium sulfate. Inhibitory effects of high levels of nitrate nitrogen were not diminished by the addition of casein-hydrolysate or by increasing the concentration of manganese in the basal medium. Furthermore, restricted growth at high concentrations of nitrate nitrogen could not be attributed to accumulation of high concentrations of nitrite nitrogen in the medium. An investigation of the influence of osmotic levels on growth revealed that *P. solanacearum* was inhibited by osmotic levels above 3.6 atmospheres. Determination of osmotic pressures of the nutrient solutions indicated that unfavorable osmotic effects may be a factor in inhibition at high concentrations of nitrate nitrogen.

Fungi associated with soybean seeds and pods, prior to harvest, at Stoneville, Mississippi, in 1952. KILPATRICK, R. A. Soybean pods of 12 varieties and strains were collected, sterilized, and the seed removed aseptically and plated. Essentially the same kind of fungi were isolated in 1952 as in 1951, although their prevalence was different. The purple-seed-strain fungus, *Cercospora kikuchii*, was not so common as in 1951, although it comprised 35 per cent of the total isolates. Dry weather appears to reduce the percentage of fungus infection in soybean seed, as none of the varieties or strains had reached 100 per cent of seeds infected prior to maturity. Pods of 21 varieties and strains of soybeans with portions of the stigma and style adhering were collected at bi-weekly intervals during the growing season of 1952. Sections of the pistil remains (1–2 mm. in thickness) were sterilized in mercuric chloride (1:1000) for 1 minute, then rinsed in 4 changes of sterile distilled-water and transferred to Petri plates containing potato-dextrose-agar. *Alternaria* spp. were most prevalent, although *Cladosporium* spp., *Fusarium oxysporum*, *Cercospora* sp., *Curvularia* spp., *Phyllosticta* spp., *Phomopsis sojae*, *Rhizopus nigricans*, *Penicillium* spp., *Aspergillus niger*, *Aspergillus* sp., and several unidentified fungi were isolated. Young pistil remains yielded fewer fungi than older ones. *Cercospora* sp., *Phyllosticta* spp., and *Phomopsis sojae* were more commonly isolated from pistil remains of pods approaching maturity.

Race 4 of the soybean downy mildew fungus, Peronospora manshurica. LEHMAN, S. G. In 1950, G. E. Geeseman published results of experiments in which he tested 9 collections of the soybean downy mildew fungus *Peronospora manshurica* (Naoumoff) Syd. on 7 varieties of soybeans. He concluded that the 9 collections represented at least 3 physiologic races. He designated these as races 1, 2 and 3 and showed that they can be distinguished by use of Richland and any one of 5 other soybean varieties which he named. Greenhouse tests made by the writer in the spring of 1952 showed that another physiologic race of the soybean downy mildew fungus exists in North Carolina. Conidia of the North Carolina race were obtained from systemically infected plants by germinating oospore-encrusted seeds of the Roanoke variety. The conidial inoculum thus obtained was increased in quantity by growing the fungus on other young Roanoke plants. Using conidia produced in this manner, inoculations were made on young plants of 20 varieties including the 7 varieties used by Geeseman. Infection readings were made on primary leaves using the severity rating system, 0, 1, 2, 3 and 4 published by Geeseman. The North Carolina race, here designated physiologic race 4, failed to infect any of the varieties that may be used in differentiating races 1, 2 and 3. In the same test, however, race 4 caused severe infection, rated in severity class 4 of Geeseman's scale, on Roanoke, Rokusun, and N48-1867. It caused mild infection, rated as severity class 1, on Acadian and Woods Yellow. No infection by race 4 occurred on Dorman, CNS, Patmetto, Ogden, Rose Non Pop, Ralsoy and N49-2370.

Reaction of the Deltapine 15 variety of cotton to different isolates of Meloidogyne. MARTIN, W. J. Root-knot nematodes were collected from 22 hosts in different parts of Louisiana. Isolates were established by placing egg masses on tomato plants, variety Rutgers, in 12-in. clay pots containing sterilized soil, and the isolates were maintained thus on tomato plants. To test the reaction of Deltapine 15 cotton to the different isolates, 2 methods were used. Cotton was interplanted with tomatoes, or cotton was grown in sterilized soil to which infective larvae of a given isolate were added. Examinations were made 60–90 days after planting. Of 40 different isolates tested, 13 failed to cause root knot on the cotton, and no evidence of larval development inside cotton roots was observed after cotton was grown in these 13 isolates. The original hosts from which the 13 isolates were obtained included tomato (4 isolates), okra (2), cucumber (1), gardenia (1), strawberry (1), hairy vetch (2), *Lamium amplexicaule* (1) and boxwood (1). Certain other isolates from tomato, okra, cucumber and hairy vetch affected the cotton and multiplied readily on it. Tentative identifications indicate that 7 of the 13 isolates which did not affect cotton were *Meloidogyne incognita*, 2 were *M. incognita* var. *acrita* and 1 was *M. hapla*.

Report of preliminary study of tri-basic copper in control of Ascochyta blight of cotton. MILLER, JULIAN H. Cotton in north Georgia, even after effective seed treatment, will often emerge to a good stand and then die in the cotyledon stage or after true leaves have developed, especially in fields where cotton has followed cotton for several years. In some fields the loss of stand was so severe that replanting was necessary. Causes of this dying were *Ascochyta gossypii* (the most common), thrips, or *Rhizoctonia solani*, either alone or in combination. To test the effect of early dusts in control, an acre farm field with a continuous cotton record was divided in plots and dusted with different treatments at 4 weekly intervals, at rate of 20 lbs. per acre. The first dust was applied when the plants were well up. Survival stand counts were made of the third row in each plot. In descending order they were 7% copper–5% DDT, 178 plants; 7% copper–2% zinc, 174 plants; 5% copper–2% zinc–5% DDT, 157 plants; 6% copper, 155 plants; check, 115 plants; cotton dust (3% BHC, 5% DDT, 40% sulfur), 110 plants. Copper was in form of tri-basic copper and zinc as zinc sulphate. All the copper dusts were significantly better in control than sulfur treatment or check, with less subsequent dying and less leaf and stem spots.

Fusarium wilt resistance and fiber properties of some new cotton hybrids and selections. NEAL, D. C. The material studied for wilt resistance was grown in an area where the incidence of wilt is high and characterized by the absence of the root-knot nematode, *Meloidogyne incognita* var. *acrita*, which attacks cotton in other areas of the South. In this particular area other parasitic nematodes, namely, *Rotylenchulus reniformis*, *Helicotylenchus* sp., and *Tylenchorhynchus* sp., occur in great abundance. Wilt resistance of several hybrids and progeny-row selections has been determined over several generations. Such fiber properties as lint percentage and staple length were determined each year and improved by continued selection. Tensile strength, while initially good for certain lines in 1948, declined in 1952. Environmental influences may be responsible. Among several hybrids studied, the more promising from the standpoint of resistance and fiber qualities are Louisiana 33, Louisiana 3314, Deltapine 6-8-5 × Delfos 6102, and Deltapine 6-8-5 × Delfos 6102 × Deltapine 6-8-5. Also showing good wilt resistance and above average fiber qualities are Coker 100-97-2-2-2, Delfos 425-920, Deltapine 6-8-5, and Stoneville 2-B-R. Louisiana 33 is now in commercial production in Northwest Louisiana and has yielded well in wilt-nematode infested areas in this section. In a field trial in 1950, Stoneville 2-B-R produced 1000 pounds of lint per acre.

Effects of soil fumigation on populations of parasitic nematodes, incidence of Fusarium wilt, and yield of cotton. NEWSOM, L. D., AND W. J. MARTIN. Soil fumigation experiments were made for controlling the wilt-nematode complex in cotton soils in Louisiana in 1951 and 1952. In 1951

control was effective in some experiments and ineffective in others. In 1952 where nematode and wilt infestations were light, soil fumigation failed to increase yields. In experiments made in soils heavily-infested with *Fusarium vasinfectum* and nematodes of the genera *Meloidogyne, Pratylenchus, Tylenchorynchus* and *Trichodorus*, soil fumigation increased yields strikingly. In 2 replicated tests in such soils, ethylene dibromide was applied in the row 4 weeks before planting the cotton variety Coker 100W. Treatments as low as 2 gal. per acre of 85 per cent ethylene dibromide gave striking responses in reduced number of wilt-affected plants, increased height of plants and increased yield of cotton. Nematode data from soil samples taken 7, 12 and 19 weeks after planting showed that there were significantly fewer nematodes of the genus *Tylenchorynchus* in treated compared to untreated plots on the first 2 dates. Numbers of nematodes of the other genera varied widely from plot to plot and were not significantly different in treated and untreated plots. However, at the end of the growing season root-knot and root-rot indices were much lower in treated plots.

Some celery seedbed diseases of central Florida and their control with certain chemicals. PERRY, V. G. AND GEORGE SWANK, JR. A complex of nematodes and fungi is described as causing considerable loss and stunting of celery seedlings in the seedbeds of central Florida. Some of these organisms may be carried from seedbeds to clean or fumigated fields on or in the roots of transplants. Species of *Rhizoctonia, Pythium*, and *Fusarium* genera of fungi have been isolated from roots of affected seedlings and used to produce symptoms of root rot and damping-off on plants grown in sterile soil. Southern blight caused by *Sclerotium rolfsii* occurs in a few of these seedbeds also. The nemic parasites most commonly found in seedbeds are *Meloidogyne* spp. (root-knot) and *Trichodorus* sp. (stubby root). The awl nematode (*Dolichodorus heterocephalus*) and the sting nematode (*Belonolaimus gracilis*) have been found in some. These nematodes are involved in the root rot complex. In several experiments soil treatments with either methyl bromide (MC-2) or chlorobromopropene (CBP emulsible) prior to planting the celery seed, has almost eliminated parasitic nematodes, parasitic fungi, and noxious weeds from seedbed areas. In all cases where either of the above materials was used, the survival and growth rate of the celery seedlings were more than twice that of untreated plots. Dichlorobutene, dibromobutene, D-D, EDB, and some other chemicals gave good control of either nematodes or fungi but were not effective against both.

Fruit rots of muskmelon in North Carolina caused by Phytophthora sp. and two sterile fungi. PERSON, L. H., AND D. E. ELLIS. In 1949 and 1950 fruit rots caused serious losses of muskmelons in the Ridgeway, North Carolina, area during excessively rainy periods just before and during harvest. The only external symptoms in early stages of the disease were slight depressions of the rind. These were not readily discernible nor constantly associated so that extensive losses of fruit that was apparently sound when packed occurred in transit. Internal symptoms were of 2 types, a colorless rot of somewhat cheesy consistency and a rather firm, light to dark brown rot. Isolation and inoculation studies showed that a fungus identified as *Phytophthora* sp. was associated with and capable of causing the colorless type rot, and that 2 apparently distinct sterile fungi with septate mycelium were capable of causing the rot with which the brown discoloration was associated. Results of inoculations indicate that all 3 fungi are capable of direct penetration of either immature or mature fruit. Observations also indicate that infection can occur through any portion of the rind whether or not in direct contact with the soil. These rots were not a problem during seasons of 1951 and 1952 which were abnormally dry.

Transmission of leaf and flower variegation in camellias by grafting. PLAKIDAS, A. G. Camellia varieties with variegated (broken color) flowers usually exhibit also varying degrees of yellow splotching of their foliage. That this color-breaking results from a virus infection has been suggested. To determine whether the leaf and flower variegation can be transmitted from one plant to another, grafting experiments have been carried on since 1950. *Camellia sasanqua* plants with 2 graftable branches were used as root-stocks. Scions from a variety with solid-colored flowers were grafted on one of the branches and scions from a variety with variegated flowers on the others. Foliage variegation has been readily transmitted from *C. japonica* to *C. japonica* (10 varieties) and to *C. sasanqua*, and from *C. sasanqua* to *C. japonica* and *C. sasanqua*. Flower variegation (color-breaking) has been transmitted from the variegated to the solid-colored variety in the following graft combinations which have bloomed to date: Mathotiana Variant, variegated × Mathotiana, solid color; Kumasaka, variegated × Kumasaka, solid color; Adolph Andusson, variegated × Adolph Andusson, solid color; Ville de Nantes, variegated × Imperator, solid color; and Empress, variegated × Empress, solid color.

Pierce's disease virus infection, a cause of grape degeneration in Florida. STONER, WARREN N. Attempts to grow grapes in Florida have all failed. Recent field and laboratory observations indicated that grape degeneration was possibly caused by Pierce's disease. Healthy vines, chip grafted in the greenhouse from known degenerate vines, in the fall of 1951 produced initial symptoms of degeneration during the summer of 1952, and by fall developed definitive symptoms similar to those of Pierce's disease. Two types of vector trials were made using *Carneocephala flaviceps* (Riley), a leafhopper related to known vectors of Pierce's disease virus. In the first, field collected insects were fed on shoots from degenerate vines and transferred to healthy grapes. In the second, insects taken on Bermuda grass near degenerate vines were fed directly on healthy grape and alfalfa plants. Symptoms of degeneration developed in the test grapes of both vector experiments. These symptoms were identical with those caused by Pierce's disease virus. The alfalfa used in the field infectivity trials developed "witches broom" symptoms, and the steles of the roots were discolored the way they would be if the plants were infected with Pierce's disease virus. The data presented indicate that Pierce's disease virus is a cause of grape degeneration in Florida, and that *Carneocephala flaviceps*, the yellow-headed sharpshooter, is a hitherto unreported insect vector of this virus.

The soil as a source of inoculum for Rhizoctonia aerial blight of Lespedeza. STROUBE, WILLIAM H. Rhizoctonia aerial blight has been reported as a serious disease of Lespedeza in the South. Observations in 1951-52 have resulted in the conclusion that this was probably the most destructive disease of Lespedeza in Louisiana. The source of inoculum and the method of initial infection were of interest. Soil samples were taken from nondiseased areas in Lespedeza fields and diluted 1:1000 with sterile water. One cc/plate of this suspension was put on poured plates of acidified water agar (pH 3.6–4.0) and incubated. Rhizoctonia was obtained from approximately 45 per cent of these plates. Eight of the selected isolates tested were indistinguishable morphologically and pathogenically from foliar isolates. Dried oat inoculum was placed on the surface of steamed soil, in which Lespedeza was being grown, and placed in a moisture chamber at high temperatures (27–30° C.). Observations at intervals showed the fungus growing up the Lespedeza stems. After 30 hours the fungus had reached the lower leaves, and after 54 hours had become established as a typical foliage blight. The results obtained indicate that the soil may serve as a source of inoculum for Rhizoctonia aerial blight, and that infection may result from fungus hyphae growing up the plant stems.

A comparison of Ascochyta abelmoschi Harter and A. gossypii Sydow in culture and inoculation experiments. THOMPSON, G. E. First generation cultures of *A. abelmoschi* and *A. gossypii* obtained from single conidia were characterized usually by greyish-black aerial mycelium when

grown on potato-dextrose agar in Petri dishes at 25° C. Certain colonies of each species were similar, while others were different. Pycnidia with conidia developed sparsely in scattered locations or in sectors in 3–4 weeks. Variants were obtained when subcultures were made of single conidia. From further subcultures additional variants developed. Strains differed from one another in color, amount of aerial mycelium, rate of growth, pycnidia production and size, color of pycnidial walls, conidia size and septation. Optimum temperature for rate of growth of certain strains of both species was approximately 25° C. Optimum temperature for growth of 1 variant of *A. gossypii* was 20° C. No distinct morphological differences could be found between pycnidia and conidia from the type specimens of *A. abelmoschi* on okra and *A. gossypii* on cotton. Inoculation of Mammoth Long Pod okra and Coker 100 wilt cotton with conidia from 1 strain of *A. gossypii* produced leaf infections on both hosts, and a die-back of cotton terminal growing points. Blue lupine was susceptible to strains of both species.

Chemical treatments of shallots for pink root control. TIMS, E. C. For several years attempts have been made to control pink root on shallot sets (bulbs) by certain chemical treatments. Pink root infected sets were soaked for varying lengths of time in chemical suspensions or solutions and planted in soil free of the disease. Among the materials tested in this way were Puratized #177 (phenyl amino cadmium dilactate) and Puratized N-5E (phenyl mercuri triethanol ammonium lactate) now known as Puratized Agricultural Spray, $HgCl_2$, Semesan, Spergon, Phygon, Ceresan, and Arasan. Results of most of the treatments were inconclusive. Usually if the chemical reduced the amount of pink root appreciably the shallot plants were severely injured. In 2 of the tests $HgCl_2$ 1–500 and Semesan 1 lb.–50 gal. showed promise, but in other tests they were not effective. A number of dust treatment tests also gave varying results. One combination liquid dip and dust treatment (Ceresan dip + Arasan dust) gave promising results in a single test. A number of tests were made in which infected shallot transplants (individual green plants obtained by dividing the bunches) were dipped or soaked in the different chemicals. Again the treatments that reduced pink root also caused severe injury to the plants.

Some results from a large-scale wheat disease survey in Oklahoma. WADSWORTH, D. F., AND H. C. YOUNG, JR. During May, 1952, a comprehensive survey of over 4500 acres of wheat was made in north-central Oklahoma. Varietal distribution and reaction to various diseases in the field are of interest. Speckled leaf blotch (*Septoria tritici*) was the most severe disease, affecting all fields. Comanche, occupying 175 acres, was the most severely affected variety, whereas Red Chief on 43 acres appeared the most resistant. Loose smut averaged 24 per cent in the variety Blue Jacket; Pawnee and Comanche had only a trace. Leaf rust was most serious on the variety Wichita, averaging 36 per cent severity (Cobb scale) at soft dough stage. Westar appeared the most resistant. Speckled leaf blotch was severe in thin stands and sparse growth, whereas leaf rust was severe in thick stands and rank growth. Growers indicated a preference for early varieties since over 50 per cent of the acreage in this area was devoted to Triumph, Early Blackhull, Wichita, and Pawnee. Less than 5 per cent of the growers interviewed recognized the difference between loose smut and bunt; many expressed disappointment with chemical seed treatment when loose smut appeared. Forty-five per cent of the wheat seed in this area was treated and 8 per cent was certified.

Toxin production in relation to tobacco black shank disease. WOLF, FREDERICK T. When *Phytophthora parasitica* var. *nicotianae* is grown in potato-dextrose broth for 4 weeks or longer, filtrates derived from such cultures induce wilting of detached leaves of tobacco and tomato. Similar results have been obtained in a number of other media, the time required for the appearance of toxin varying with the composition of the medium. The toxin is almost quantitatively removed from filtrates by adsorption on charcoal, is not precipitable from solution by protein precipitants such as alcohol or magnesium sulphate, is ether insoluble, dialyzable, and partially heat stable. Samples of sap from tobacco (var. Hicks) were prepared from healthy and from black shank diseased plants, a portion of the sap from diseased plants being treated with charcoal. All samples induced wilting of tobacco and tomato leaves within 1 hour. The wilted leaves were then placed in distilled water, whereupon both tobacco and tomato leaves recovered following exposure to healthy tobacco sap, and to diseased tobacco sap (charcoal treated), but not following exposure to untreated sap from diseased plants. This demonstrates that toxin production occurs in diseased plants as well as in cultures of the fungus. Further studies concerned with the properties of the toxin are in progress.

Collection and race isolation of wheat leaf rust in Oklahoma. YOUNG, H. C., JR., AND D. F. WADSWORTH. During 1950, leaf rust collections placed in glassine bags in the usual manner and refrigerated at 2°–4° C. produced viable urediospores in only 68 per cent of the collections after 4–5 months storage. When leaf rust samples were collected in 1951, each sample was placed in a folded blotter which was clipped together and dropped into a test tube containing approximately 2 gm. of calcium chloride. The blotters were held in the tubes for 30 days, then placed in manila envelopes until needed. Both tubes and envelopes were refrigerated at 2°–4° C. By desiccating the samples immediately after collection, 99 per cent contained viable urediospores, even after 7 months storage. In both 1950 and 1951, 4 races (5, 15, 105 and 9 in order of their abundance) constituted over 85 per cent of the race population. Several collections of race 105 proved capable of attacking the variety Westar and many other Hard Federation derivatives which are normally resistant to this race. These biotypes are important since several Hard Federation hybrids (Westar, C.I. 12517, and C.I. 12515) have been superior in yield in uniform tests throughout the southwestern hard red winter wheat region.